GUSTAVO DIAS DE ALMEIDA

QTL MAPPING REVEALS CONSTITUTIVE AND ADAPTIVE GENOMIC REGIONS FOR DROUGHT TOLERANCE IN TROPICAL MAIZE (*Zea mays* L.)

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 17 de julho de 2012.

Dr. Lauro José Moreira Guimarães

Dra. Claudia Teixeira Guimarães

Dr. Raman Babu
(Coorientador)

Prof. Cosme Damião Cruz
(Coorientador)

Prof. Aluízio Borém
(Orientador)

"It is not the strongest of the species who survive, nor the most intelligent, but the one most responsive to change"

Charles Darwin

DEDICO

Aos meus pais,

***José Elias de Almeida**, que com o suor do seu rosto fez brotar da terra o sustendo de nossa família;*

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BIOGRAPHY

GUSTAVO DIAS DE ALMEIDA, son of José Elias de Almeida and Marlene Dias Almeida, born in Barra de São Francisco City, Espírito Santo State-Brazil in 04th April, 1985.

In 2003, he started the college of Agronomy at Universidade Federal de Viçosa (UFES) getting degree in July of 2007.

In August 2007, he joined the graduate program in Crop Science at Universidade Federal de Viçosa (UFV). He then obtained his Master's degree at UFV in February 2009. In March 2009, he joined in Ph.D. program in Genetics and Breeding in Universdiade Federal de Viçosa under the guidance of Prof. Aluízio Borém. He went to develop his thesis research in CIMMYT-Mexico under supervision of Dr. Raman Babu and receiving PhD degree in July 2012.

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ABSTRACT

ALMEIDA, Gustavo Dias, D.Sc., Universidade Federal de Viçosa, July, 2012. **QTL mapping reveals constitutive and adaptive genomic regions for drought tolerance in tropical maize (*Zea mays* L.)**. Adviser: Aluízio Borém. Co-advisers: Raman Babu and Cosme Damião Cruz.

Drought is the most important abiotic stress resulting in significant yield losses in maize (*Zea mays* L.). Maize now recognized as one of the major and eminent food security crops due high yield potential compared with another important crops as rice and wheat. Development of more drought-tolerant genotypes can contribute to ensure food security mainly in developing areas in Africa, Asia and Latin America where this crop is a staple food. However, selection for drought tolerance is difficult because it is a complex trait with strong interactions between genotypes and environments and limited knowledge about the role and regulation of tolerance mechanisms. Classical breeding have identified morphophysiological traits related to grain yield under drought conditions. Most of these traits are polygenic, but grain yield probably remains the most polygenic and complex trait. The availability of molecular markers allowed mapping Quantitative Trait Loci (QTLs). It is a promising tool for detection constitutive and adaptive genomic regions controlling drought tolerance as well as for studying changes in the expression of these loci across varying environmental conditions. These genomic regions may be considered target for markers-assisted selection (MAS) program to develop drought tolerant genotypes. We evaluated three tropical maize populations from CIMMYT's Global Maize Program under water stress (WS) and well-watered (WW) regimes in Mexico, Kenya and Zimbabwe to provide an understanding of the genetic basis of yield and secondary traits involved in response to water-limited conditions at flowering time. To achieve this goal we conducted QTL mapping studies in single and multiple environments as well as a meta-QTL analysis to identify the most prone genomic regions across populations to be useful in MAS program. Grain yield (GY) and anthesis-silking interval (ASI) were measured in Mexican and African environments, while secondary traits, as ears per plant (EPP), stay-green (SG) and plant and ears height (PEH) were realized only in Mexican environments. Drought stress reduced GY around 50% and increased ASI above 80%. Interestingly another morphophysiological traits like EPP, SG and PEH

were not markedly affected by water shortage. In general drought stress tends to reduce genetic variance of GY, while secondary traits remain to be stable or even higher under water scarcity. Also, high correlation between morphophysiological traits and GY were observed under drought condition. Grain yield QTLs showed strong interactions with the environment (QEI) and changed their positions on the genome across environments. Whereas QTLs for secondary traits tend to be more stable across water regimes. Meta-QTL analysis reveals clusters of QTLs for grain yield and secondary traits, such as anthesis-silking interval, ears per plant, stay-green and plant and ears heights on chromosomes 1 (bin 1.06 at 161.07-183.83 Mb) and 10 (bin 10.04-06 at 111.26-141.82 Mb) while one interesting cluster of all secondary traits were detected on chromosome 3 (bin 3.06 at 169.75-178.23 Mb) under both water regimes. The confidence interval of metaQTLs regions harbored several genes, available in maize database (http://www.maizegdb.org), that were involved in diverse networks controlling development, metabolism and responses to biotic and abiotic stresses. The target regions identified by QTL mapping can contribute to complementing the evaluation and selection of improved germplasm, especially in poor areas with high risk of drought as in sub-Saharan Africa.

RESUMO

ALMEIDA, Gustavo Dias, D.Sc., Universidade Federal de Viçosa, julho de 2012. **Mapeamento de QTL relevela regiões genômicas constitutivas e adaptativas para tolerancia à seca em milho tropical (*Zea mays* L.)**. Orientador: Aluízio Borém. Coorientadores: Raman Babu e Cosme Damião Cruz.

A seca é o estresse abiótico mais importante e resulta em prejuízos significativos na produtividade do milho *(Zea mays* L.). Atualmente o milho é reconhecido como uma das principais culturas agrícolas respónsaveis pela segurança alimentar devido ao alto potencial de rendimento comparado a outras culturas como arroz e trigo. O desenvolvimento de genótipos tolerantes à seca pode contribuir para garantir a segurança alimentar, principalmente em países em desenvolvimento da África, Ásia e América Latina, onde essa cultura é considerada alimento básico. No entanto, a seleção para tolerância à seca é difícil devido à complexidade dessa caracteristica, alta interação entre genótipos e ambientes e o conhecimento limitado sobre o papel e regulação de mecanismos de tolerância. Melhoramento genético clássico têm identificado características morfofisiológicas para a produção de grãos sob estresse hídrico, mesmo que sejam em sua maioria poligênica. No entanto, rendimento de grãos, provavelmente, continua sendo a característica mais complexa. A disponibilidade de marcadores moleculares tem permitido o mapeamento de *Quantitative Trace Loci* (QTLs). O qual é uma ferramenta promissora para detecção de regiões constitutivas e/ou adaptativas que controlam a tolerância à seca, bem como para entender as alterações na expressão destes loci entre diferentes condições ambientais. Essas regiões genômicas, podem ser consideradas alvo para programas de seleção assistida por marcadores moleculares (MAS) no desenvolvimento de genótipos tolerantes à seca. Foram avaliadas três populações de milho tropicais desenvolvidas pelo CIMMYT, em condições hídricas normais (WW) e de estresse hídrico (WS) no México, Quênia e Zimbábue para estudo da base genética de rendimentos de grão e características morfofisiológicas envolvidas na resposta ao estresse hidrico durante período de floração. Para atingir este objectivo foram realizados estudos de mapeamento de QTL em simples e múltiplos ambientes, bem como uma meta-QTL análise para identificar as regiões genómicas estáveis entre

populações, e portanto mais promissoras em programas de seleção assistida por marcadores. O rendimento de grãos (GY) e intervalo de florescimetnos feminino e masculino (ASI) foram mensurados em ambientes Mexicanos e Africano, enquanto que características secundárias como o números de espigas por planta, *stay-green* e alturas da plantas e de espigas foram realizadas apenas no México. O estresse hídrico claramente reduziu o GY cerca de 50% e aumentou a ASI acima de 80%. Interessantemente, outros caracteres secundários, como EPP, SG e PEH não foram significativamente afetados pelo estresse hídrico. Em geral, o estresse hídrico tende a reduzir a variância genética de GY, enquanto que para características morfofisiológicas a variância genética pode se mostrar estável ou mesmo superior em condições de seca. Além disso, correlações significante entre características morfofisiológicas e GY foram observados. QTLs para produtividade de grãos mostraram fortes interações com o ambiente (QEI). Enquanto que QTLs para características secundárias tenderam ser mais estáveis entre nos regimes hídricos. A meta-análise revelou clusteres de QTLs para produtividade de grãos e características morfofisiológicas, como o intervalo de florescimento, espigas por planta, *stay-green* e alturas de plantas e espigas, principalmente, nos cromossomos 1 (bin 1.06 em 161.07-183.83 Mb) e 10 (bin 10.04-06 em 111.26-141.82 Mb), enquanto um cluster de todas as características secundárias foi detectado no cromossomo 3 (bin 3.06 em 169.75-178.23 Mb) sob ambos os regimes hídricos. O intervalo de confiança dos metaQTLs abrigou vários genes, disponíveis no banco de dados de milho (http://www.maizegdb.org), que estão envolvidos em diversas vias que controlam o metabolismo, desenvolvimento e respostas à estresses bióticos e abióticos. As regiões genômicas alvo identificados pelo mapeamento de QTLs podem contribuir para complementar a avaliação e seleção de germoplasma melhorado, especialmente em regiões pobre sujeitas à seca como a África subsaariana.

1. GENERAL INTRODUCTION

1.1 Drought: Its effects in human society

In plants drought stress occurs when the loss of water by the transpiration exceeds the root capacity of absorbing water from the soil for time sufficient enough to cause irreversible damage to the plant (Jaleel et al. 2007). When the water deficit occurs before the crop is fully developed, it can also reduce vegetative growth. The negative impact of drought depends mainly on the timing, duration and intensity of the stress. However, the occurrence of natural drought is largely unpredictable, making it difficult or almost impossible to distinguish between water-limited and non-limited agricultural systems (Messmer 2006).

Low water availability is one of the major causes for crop yield reductions affecting most farmed regions around the world (Ali et al. 2011). The large negative impact of drought in agriculture has been associate to the collapse of many ancient civilizations (Cázares et al. 2003). Historical evidence links drought to the collapse of the Mayan civilization from around 910 to 760 B.C. The first phase occurred between 810 and 760 B.C., the second phase was largely over by about 860 B.C. and the third and final phase finished around 910 B.C. These followed drought cycles in the Yucatan peninsula (Southern Mexico, Belize and Guatemala) strongly reduced the maize yield capacity, the main food of Mexican ancient civilizations, not providing sufficient food to about 13 million of people of the Maya society during the classic period (950 to 250 B.C.) (Huang et al. 2003).

The catastrophic droughts damaged agricultural production and caused a regional collapse. This lack of rain was also responsible to the collapse of other ancient society. In Mesopotamia, a canal-supported agricultural society collapsed after a severe 200-year drought about 3400 years ago. Also, drought appears as the

main reason for the decline of Mochica culture in coastal Peru about 1500 years ago and it is related to the end of Tiwanaku culture in Bolivian-Peruvian Altiplano about 1000 years ago (Peterson and Huang 2005).

In the modern society, drought events have been related with a tremendous impact over millions of people around the world. A water shortage in northern China in 1876 dried up crops in an extensive region, producing famine and millions of deaths. In the USA, the most well-known American dry-period was the "Dust Bowl" on the Great Plains from 1931 to 1936. The years of 1934 and 1936 were the two driest years in the history of U.S.A. climate (Lassieur 2009). Russia also experienced a severe period without rain in 1890 and 1921, the later resulted in to five million deaths in the Volga river basin. The Northeast of Brazil is a semi-arid region. The lack of rain is the reason of exodus of many families from this region to others parts of Brazil. Drought has been reported in the Brazilian literature in a novel called barren lives (*Vidas Secas*) write by Graciliano Ramos in decade of 1930 that related the story of a poverty-stricken family (Fabiano, the father; Sinhá Vitória, the mother; two sons (just called boys) and their dog called Baleia) escaping from a severe drought.

According to the World Health Organization, drought is the causes of death for about half the people who are killed by natural disasters (Cázares et al. 2011). Therefore, drought will continue being a problem to be solved for agriculture production. Adversely, the scenario of the global climate changes will probably aggravate the issues of food insecurity, hunger and malnutrition for millions of people, particularly in southern Asia, sub-Saharan Africa and Latin America (Varshney et al. 2011). Moreover, it has been projected that in 2050 the world population will reach about 9 billons of people, which represents an increase of 70% in the demand of the food production in the world (Gilbert 2010). This scenery is

driving plant scientist to breed plants that can be grown in marginal areas, as those with limited rainfall (Hao et al. 2010).

1.2 Maize: Origins, classifications and importance

Maize or corn is a grass from the Poaceae family, taxonomically classified as *Zea mays* L. spp. *mays*, domesticated possibly around 9000 years ago by Mexican ancient habitants. Species of Maydae tribe are characterized for monoecism, in which flowers are unisexual with male and female inflorescences almost one meter apart on the same plant while other grasses from this tribe have hermaphrodite flowers. Maize is a worldwide crop, however the origin of this cereal is essentially American because in this continent are found wild close relatives (Tesosinte and *Tripsacum*) were found as well as fossil, historical and linguistic evidence of the domestication of this crop. The most accepted hypothesis for the origin of maize is that this grass was derived from the wild plant called teosinte (*Zea mays* spp.) which is composed of the following sub-species: *Z. mays* spp. *mexicana*, *Z. mays* spp. *parviglumis*, *Z. mays* ssp. *luxurians* and *Z. mays* spp. *diploperenis.* Both maize and teosinte have the same number of homologs chromosomes (n=10), it can easily cross resulting in a fertile offspring with genetic similarity to both parents (Paterniani and Campos 2005).

Maize can be considerate a gift from the New Continent to the world because the Old World only had contact with this crop after the discovery of the American continent. In Africa, for example, maize was introduced by the Portuguese explorers in the beginning of the 16th century (Ganunga 2005). Currently, maize is the most important economic crop in the world and now it has been recognized as a major and eminent food security crop worldwide due the high yield potential rather than other crops, as wheat and rice (Ge et al. 2012). The successful and continuous production of

maize could be a key to global food security since. Because this crop can be a direct food source for human nutrition as well as for animal feed in many tropical and sub-tropical zones. This crop is a staple food in many regions in the world, mainly in developing countries (Araus et al. 2011). In Africa, maize accounts for one fifth of the total daily calorie intake per capita, at least, in 12 countries and for more than 50% in countries like Lesotho, Zambia and Malawi. In West African coastal countries, the estimated maize per capita consumption ranges from 41 to 102 kg per year (Krivanek et al. 2007). The demand for maize in developing countries is expected to exceed 500 million tons in 2020, surpassing the demand for both rice and wheat. This projected rapid increase in demand is mainly explained by the increase in the demand for maize as livestock feed (for poultry and pigs, particularly in eastern and southeastern Asia) (Araus et al. 2011). In addition, maize has an important role in countries facing food shortage because this crop is grown over a wide range of environmental and geographical regions including lowland, mid-altitude and sub-tropical highland environments (Zaidi 2004).

1.3 Aspects of drought tolerance in maize

The water efficiency in crops shall also be increased, particularly in rainfed agriculture systems. The resulting benefits are twofold: On the one hand, food security in rainfed systems will improve and on the other, the overall water balance will be more favorable. The more food produced in rainfed systems, the smaller demand for freshwater resources for irrigation (Messmer and Stamp 2010). The benefit of genetic improvement of water deficit could strongly help poor farmers, mainly, by stabilizing their yield once tolerant genes are incorporated into the seed,

therefore, they will no longer depend on agronomic techniques to have high yield productions (Duvick 2005; Bänzinger et al. 2006).

The response of the crop to stress depends on numerous traits, many of which are constitutive, but which may also be modified by stress. A drought tolerant genotype produces higher yields than a drought susceptible genotype in a variety of water-stressed environments. The ideal genotype combines both high yield under favorable conditions and tolerance to water stress. The overall goal of breeding for drought tolerance is, therefore, to realize a high maximum yield potential and to reduce the gap between yield potential and yield under stress (Ali et al. 2011).

The success of breeding programs for drought tolerance requires knowledge of the morphophysiological aspects related to drought stress. It is necessary the understanding on the critical phase of the culture, parts of the plant affected most by the drought and if there is any trait correlated with grain yield under stress condition (Bänzinger et al. 2000). A severe drought stress tends to reduce the genetic variance of the grain yield in maize (Bänzinger et al. 2000). Further, there is a high genotype environment interaction (GEI) between drought and well water environments for the genotype performance which reduces the odds to selected genotypes with good performance under both water regimes (Bänzinger et al. 2006). Breeding programs for drought tolerance as from CIMMYT (*Centro International de Mejoramiento de Maíz y Trigo*) has been adopting some secondary traits in the breeding process. These traits should be genetically correlated with grain yield in the target environment, they also have to be genetically variable with a high level of heritability, they should be simple, cheap, non-destructive and easy to be assessed, be stable throughout the measurement period and they should not be associated with any yield loss under non-stressed conditions (Ribaut et al. 2009)

About 50% of the on-farm increase in the yield of hybrid maize during the last 60 to 70 years has been achieved through genetic improvements, mainly by the better tolerance to stress that has been incorporated into newer hybrids. The increase in yield was accompanied by changes in a number of morphological and physiological traits, as for example anthesis-silking interval (ASI), stay-green, logging resistance, plant height and more efficient photosynthesis, as well as an improved photosynthetic rate after stress events (Duvick 2005). Considerable progress in the genetics of tropical maize was also achieved, leading to a higher yield potential and to an improvement in drought tolerance (Bänziger et al. 2000; Monneveux et al. 2008).

All maize growth stages are affected by the drought, but the crop is extremely sensitive in the period from -2 to 22 days after silking emergence, with a peak at 7 days, and almost complete barrenness can occur if maize plants are stressed in the interval from just before tassel emergence to the beginning of grain fill (Bolaños and Edmeads 1996; Bänzinger et al. 2000). When photosynthesis at flowering is reduced by drought stresses, silk growth is delayed, leading to an easily measured increase in the anthesis-silking interval (ASI) and kernel abortion. Dry periods during this time results in one considerable increase in the anthesis-silking interval (ASI) due the delay in silking date (SD). It happening due the fact that to silks growth and kernel number depends directly on the flow of photosynthetic products during the three weeks of extreme sensitivity bracketing flowering. Also, this crop is thought more susceptible at flowering than other rainfed crops because male and female flowers are separated by as much as one meter, and pollen and fragile stigmatic tissue are exposed to a dry and otherwise hostile atmosphere for pollination to occur (Bänzinger et al. 2000). Breeding for drought tolerance at flowering, the developmental stage, at which maize is most susceptible to drought, has identified key secondary traits for grain yield, with

the anthesis-silking interval being the most prominent one (Bolaños and Edmeades 1996; Ribaut et al. 1997; Bänzinger et al. 2000; Campo et al. 2004; Messmer et al. 2009).

Low values of ASI indicates synchronism between male and female flowering time, resulting in an adaptation to stress which can provide a better grain yield under the adverse water condition. The anthesis-silking interval has been considered a major secondary trait used in breeding program for drought tolerance (Beltrán et al. 2003). Because of the stress, this trait shows an increase in the genetic variances, with the heritability ranging from medium to high and also a high genetic correlation with yield (Bänzinger et al. 2000). Messmer et al. (2009) demonstrated that the heritability of ASI increased from 0.52 in well-water to 0.75 in water stress conditions. Bolaños and Edmeades (1996) found significant genetic correlation of the -0.60 of between ASI and yield under stress conditions whereas Ribaut et al. (1997) detected a non-significant correlation of -0.07 between these two traits under optimal water conditions. Even though ASI is a complex trait which torn the selection for this trait also complicate (Buckler et al. 2009), this trait has been widely used in breeding process to drought tolerance.

Further, when the stress is too strong, it leads to a long ASI, inhibiting the silk emission, and it may drastically reduce the number of ears per plant (EPP). In this situation, EPP is an important trait measured in breeding program to drought tolerance because plants with more than one ear can be more stable under stress conditions (Bänzinger et al. 2000). Moreover, EPP is easily measured, shows a high heritability and it is correlated with yield under water restrictions conditions. Positive genetic correlations of 0.70 (Bolaños and Edmeads, 1996) and 0.96 (Monneveux et al. 2006) between EPP and yield were detected under water stress during the flowering period.

Photosynthesis can be reduced by the drought stress by reactive oxygen species or by the remobilization of nitrogen from the older leaves in response to a decreased N-uptake by the roots (Rivero et al. 2007). This nitrogen is remobilized from chloroplast to the ears developing. The disintegration of the chloroplasts, a carrier of the photosynthetic apparatus, results in the typical yellowing of the leaves, a symptom which is commonly referred to as senescence (Messmer et al. 2011). The tolerance to premature senescence is referred to as stay-green, which is characterized by the increase of the photosynthesis capacity at the final stage of the plant cycle, providing a greater amount of photo-assimilates available for grain filling (Camara et al. 2007). A genotype can be considered as being stay-green if the time of contributions from green tissues is higher than the average of the population and the kernel moisture should be equal to or below the average of the population. Because if one genotype displays green leaves for a longer time but presents kernel moisture higher than the average of the population, it indicates that this genotype is not considered as stay-green but a genotype with a longer vegetative period (Belicuas 2009). Moreover, the stay-green trait was not found as being associated with a decrease in yield under normal water availability (Messmer et al. 2011).

Stay-green can be directly measured by a visual scale from 1 to 10, considering how much of the leaf area is dead, therefore 1 (10%), 2 (20%), 3 (30%), 4 (40%), 5 (50%), 6 (60%), 7 (70%), 8 (80%), 9 (90%) and 10 (100%) (Bänzinger et al. 2000). However, an accurate phenotyping is essential for phenotypic or molecular selection in any breeding program. Leaf longevity is related with the nitrogen status in this part of the plant. Thus, the chlorophyll content in leaves is a strong indication of delayed leaf senescence (Kassahun et al. 2010). It seems that it is easy to quantify the stay-green trait by measuring the relative leaf chlorophyll content

with a portable chlorophyll meter (SPAD meter, Konica Minolta Inc). The SPAD values provide an indication of the relative amount of total chlorophyll (mg/cm^2) in the leaves. Leaf chlorophyll content was reported to be positively correlated with grain yield (Xu et al. 2000).

1.4 Genetic mapping and QTL detections

Molecular markers is a recent approach in breeding programmes, however the theory of the Quantitative Trait Loci mapping, popularly known as QTL, was first described in Sax in 1923. This author noted that seed size in common bean (*Phaseolus vulgaris*), which is a complex trait, was associated with seed coat color, a monogenic trait. He interpreted this finding as the link of the single gene controlling seed color with one or more of the polygenes controlling seed size. The idea was that if segregation of a single gene marker could be used to detect and to estimate the effect of a linked polygene and if single gene markers were scattered throughout the genome of an organism, the mapping and characterizing of polygenes affecting a character (Tanksley 1993) would be possible. This idea is fascinating because quantitative traits are determined by several genes (polygenes) with small effects, highly influenced by the environment and usually with low heritability, which increases the work of the breeder (Falconer 1998).

The first genetic maps were based on morphological and cytological markers of the discrete traits. With the advent of molecular markers in the 1980s, the same idea was used, with the key innovation being that defined sequences of DNA act as the linked monogenic markers (Young 1996). This has the profound effect of driving the focus in studies of polygenic traits to questions about the chromosomal locations,

the actions of the gene, and on the biological roles of specific loci involved in complex phenotypes.

Molecular markers can be classified into two distinct classes: random marker and linked markers. Random markers are used in fingerprinting and diversity studies. Linked markers are those linked to the useful trait, which are the focus for marker-assisted selection approaches (SAM) that could be used to accelerate the breeding process. Individuals in a suitable mapping population (F_2, recombinant inbred lines, backcross,) are analyzed in terms of DNA marker genotypes and of the target phenotype. For each DNA marker, the individuals are split into classes according to marker genotype. Mean and variance parameters are calculated and compared among the classes. A significant difference between classes suggests that there is a relationship between the DNA marker and the target trait, that is an evidence that DNA marker is probably linked to a QTL (Tanksley 1993).

Many studies with the objective of finding Quantitative Trait Loci (QTL) have been conducted to identify genomic regions responsible for traits and that could be applied in breeding programmes. The effects and locations of marker-linked genes that have an impact on a number of quantitative traits could be estimate by using an approach that could be applied to dissect the genetic make-up of any physiological, morphological and behavioral trait in plants (Vinod 2009). Molecular markers allows the genetic dissection of the quantitative traits in Mendelian factors controlling the adaptive or constitutive response of crops to abiotic stress (Guo et al. 2008).

The molecular markers are highly polymorphic and their heritage was not influenced by the environment, further they are able to detect small variations, especially with the development of the Single Nucleotide Polymorphism (SNPs) (Shirasawa et al. 2010). SNPs markers are variations of nucleotides or small

insertions/deletions (indels) in sequences of bases in DNA homologous fragments and are considered the most valuable markers for genetic mapping and association studies (Zhu and Salmeron 2007). SNPs are the most common polymorphism among individuals of any species (Deschamps and Campbell 2010) because they are highly polymorphic, evenly distributed, co-dominant, accurate, reproducible, high-throughput and cost-effective (Trebbi et al. 2011). The availability of SNP genotyping platforms has been facilitating studies which include genetic diversity analysis, linkage map construction and QTL mapping for dissection of the genetic traits and the application of marker-assisted and genomic selection (Wen et al. 2011). According to Tenaillon et al. (2001) in maize, the average of one SNPs is every 104 pb between two randomly sampled sequences. Its torn this molecular marker valuable for QTL mapping studies in maize.

Quantitative Trait Loci mapping has become a standard procedure to study the genetic architecture of quantitative traits because it allows the estimation of the QTL number, their genomic position and the genetic effects of the QTL that control complex traits (Wang et al. 2010). Moreover, the molecular markers are also useful in the analysis and interpretation of the cause-effect relationship among the traits (Malosetti et al. 2008), which is a prerequisite to allow cost-effective applications of genomics-based approaches to breeding programs aimed to improving the sustainability and stability of yield in abiotic stress conditions (Collins et al. 2008).

Drought tolerance is considered a complex trait regulated by several genes. In relation to plant breeding, one of the major applications of the QTL mapping is the decomposition of complex genetic traits into their Mendelian components and the possibility of using this information in breeding programs (Bernardo 2009). Detection of a marker linked to a characteristic of agronomic interest allows selection of

genotypes on the basis of the marker phenotype. It is important, especially in cases in which the evaluation of the trait of interest is time-consuming and costly, especially for phenotypic traits with low heritability (Carter et al. 2010).

Extensive genetic dissections of drought tolerance traits have been carried out in maize, resulting in numerous QTL involved in the determination of morphological traits, yield components, flowering traits and plant height traits. Marker-assisted selection (MAS) has been successful used for simple traits controlled by only few major genes. However, for complex agronomic traits such as yield under drought stress MAS strategies have contributed less to improving germplasm than initially thought (Ribaut et al. 2009). Some limiting factors for MAS of complex traits were the specificity of QTLs to either stress or non-stress conditions, the low percentage of phenotypic variance explained by the individual QTLs, the cross-specificity of the QTLs and their sensitivity to changing environmental conditions (Campos et al. 2004).

The differential expression of the phenotypic trait by genotype across environment (GEI) is an old problem of primary importance for quantitative genetics (Falconer et al. 1989). The GEI can drastically change the ranking of the genotypes in different environments, which is a major challenge to breeders to obtain fast genetic progress (Peng et al. 2011). The advances in molecular technology and in the methods for detecting QTL have made possible to break down GEI into its QTL x environment interactions (QEI) (Chen et al. 2010). Overall, most of the QTLs identified for yield components and other quantitative traits could explain only a small percentage of phenotypic variation (often less than 10%) and could not be repeated in different environments and populations. However, studies to identify QTLs for drought tolerance have found same stable QTLs across the same water management, but few

QTLs had been expressed in well-water and stress conditions (Ribaut et al. 1997; Malosetti et al. 2008; Messmer et al. 2009; Hao et al. 2010; Zhu et al. 2011). The understanding of GEI and QEI components is essential to lead to a breakthrough in breeding under drought conditions and to the use of marker assisted selection (Li et al. 2011). QTLs stable across environments are crucial to develop varieties with large potential of grain yield in stress and well-watered conditions (Messmer et al. 2009).

Introgression of QTLs with major effects for grain yield under drought could be efficient for reduce the time to breeding tolerant maize varieties (Ribaut and Ragoto 2007, Messmer et al. 2009). However, the differential expression of QTL across environments and genetic backgrounds is an old issue for MAS. Most of the mapped QTL for grain yield under drought usually are mapped in early segregating generations, evaluated in a limited number of environments and in single genetic background, which may not provide a consistent effect because of the variation in the genetic background and environment (Truntzler et al. 2010). Additionally, the epistatic interactions can reduce or even inhibit the QTL effects in a new genetic background (Collins et al. 2008). Based on this, a more efficient way to select QTL for MAS is by comparing the consistency of the identified QTL across environments and effect across genetic backgrounds. Consistently identified QTL in the same chromosomal location, explaining high phenotypic variance with a major effect on a trait can be effectively used in MAS, fine mapping, candidate gene identification, and functional analysis (Swamy et al. 2011). Integration of QTLs detected across environments and genetic background could be realized through meta-QTL analysis and true QTL with more accurate confidence intervals and small target regions for candidate genes could be revealed (Goffinet and Gerber 2000; Swamy et al. 2011).

Also, it is important to consider in this study that even the most sophisticated tool for data analysis cannot compensate for unsound phenotyping. Precise phenotyping remains the critical and the most important step in practical breeding as well as in studies combining physiology and genetics with the objective of dissecting the plant responses to stress conditions, especially in field conditions.

1.5 Goals and objectives

The overall goal of this study was to provide a good understanding of the physiological and genetic mechanisms of drought tolerance in three tropical maize populations growing in different stress environments by QTL analysis of grain yield and morphophysiological traits in order to complement the evaluation and selection of improved germplasm. Stable genomic regions across environments and genetic backgrounds were identified. These regions are physically delimited by a set of SNPs which allow the detection of precise genomic regions at a physical position on genome for yield components and morphophysiological traits. Also we provide a understanding of traits relationship at molecular level. In addition, this approach enables to integrate marker assisted selection for drought tolerance in the conventional maize improvement programs for the tropics.

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CHAPTER I [1]

QTL mapping in three tropical maize populations reveals a set of constitutive and adaptive genomic regions for drought tolerance

Abstract: Despite numerous published reports of Quantitative Trait Loci (QTLs) for drought related traits, practical applications of such QTLs in maize improvement are scarce. Identifying QTLs of sizeable effects that express more or less uniformly in diverse genetic backgrounds across contrasting water regimes can complement significantly the conventional drought tolerance breeding efforts. We evaluated three tropical bi-parental populations under water stress (WS) and well-watered (WW) regimes in Mexico, Kenya and Zimbabwe to identify stable genomic regions responsible for grain yield (GY) and anthesis-silking interval (ASI) across multiple environments and diverse genetic backgrounds. Across the three populations, on an average, drought stress reduced the GY by more than 50% and increased the ASI by 3.2 days. We identified a total of 83 and 62 QTLs through individual environment analyses for GY and ASI, respectively. In each population, most QTLs consistently showed up in each water regime. Across the three populations, the phenotypic variance explained by various individual QTLs ranged from 2.6 to 17.8% for GY and 1.7 to 17.8% for ASI under WS environments and from 5 to 19.5% for GY under WW environments. Meta-QTL analysis across the three populations and multiple environments identified seven genomic regions for GY and one for ASI, of which six

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mQTLs on chr.1, 4, 5 and 10 for GY were constitutively expressed across WS and WW environments. mQTL on chr.7 for GY and one on chr.3 for ASI were found to be 'adaptive' to WS conditions. High throughput assays for SNPs delimiting the physical intervals of these mQTLs have been developed. At most of the QTLs, almost equal number of favorable alleles was donated by either of the parents within each cross, thereby demonstrating the potential of DT x DT (drought tolerant x drought tolerant) crosses to identify QTLs under contrasting water regimes.

Keywords: drought tolerance, SNP, QTL x Environment Interaction, metaQTLs.

Introduction

Drought is one of the most important constraints of global agriculture and severely affects maize, the most important African staple food crop. Three-quarters of the world's severe droughts over the past 10 years have occurred in Africa, which is evidenced from an extremely variable aggregated regional production that ranged from 7.6 to 22.7 million tonnes, in close correlation with the rainfall (Banziger et al. 2006). Though drought affects maize at almost all growth stages, the crop is extremely sensitive in the period from one week before to three weeks after flowering time (Banziger et al. 2000). Maize is widely regarded to be more susceptible to drought at flowering than other rain-fed crops because of a combination of several factors such as physical separation of male and female flowers, floral asynchrony, non-receptivity of the silk, tassel blasting, trapped anthers and embryo abortion (Westgate and Boyer, 1985, Lu et al. 2011). Consequently, breeding maize for reproductive stage drought tolerance holds great potential to obtain improved varieties that can withstand varying degree of water stress (Bolaños and Edmeades 1996; Ribaut et al. 1997; Messmer et al. 2009; Zhu et al. 2011).

Duvick et al. (2005) estimated the rate of breeding progress for temperate maize germplasm under mild drought to be 0.85% per year for hybrids released between 1930 and 1990, and slightly less under optimal conditions. The significant breeding gain in temperate maize under drought stress is mainly attributed to the use of rain-fed breeding nurseries with high plant densities and large scale multi-location testing (Banziger and Araus, 2007). Plant water and nutrient deficits occur more readily under high plant densities and the large-scale multi-location testing frequently exposed newer hybrids to drought conditions (Tsonev et al. 2009). With the introduction of 'managed stress' screening, especially for reproductive stage drought tolerance, a higher breeding progress of 2 – 2.5% per year was reported (Campos et al. 2004). Despite the shorter breeding history, yield gains of 3.8% to 6.3% per year under drought and slightly less under optimal conditions were reported for tropical maize (Banziger and Araus, 2007), which were mainly associated with increased flowering synchronization, fewer barren plants, a smaller tassel size, a greater harvest index, and delayed leaf senescence (Ribaut et al. 2009).

Tolerance to drought in maize is a polygenic trait and typically has low heritability and characterized by high Genotype x Environment Interaction (GEI). Conventional breeding based on direct selection of phenotypes under drought has led to impressive yield gains in maize but underlying genetic causes largely remain unknown. Quantitative trait loci (QTL)-based approaches can contribute significantly to the understanding of genetic basis of crop performance especially under drought stress conditions and such knowledge may be crucial in designing cost-effective breeding approaches aimed at improving sustainability and stability of grain yield under adverse conditions (Collins et al 2008).

In maize, QTL mapping for grain yield (GY) under water stress and other associated traits such as anthesis-silking interval (ASI) in maize has been an active area of research especially in the past two decades. The QTLs detected under water stress and well watered conditions can be categorized according to the stability of their effects across environmental conditions. A 'constitutive' QTL is consistently detected across most environments, while an 'adaptive' QTL is detected only in specific environment such as WS conditions (Collins et al. 2008). One of the earliest studies involving tropical germplasm under managed stress conditions identified 13 QTLs on chromosome 1, 2, 4, 6, 7, 8 and 10 for grain yield, of which QTLs on chr.1 and chr.10 were stable across WW and WS environments (Ribaut et al. 1997). Since then, a number of QTLs regulating morphophysiological component traits as well as GY have been reported in maize (Malosetti et al. 2008; Messmer et al. 2009; Li et al. 2010; Messmer et al. 2011). An updated compilation of mapped QTL and major genes associated with abiotic stress tolerance including drought in maize is available at www.maizegdb.org as well as www.plantsress.com. Drought tolerance QTL studies in maize and other crops and the strategies for their use in marker assisted selection (MAS) in breeding programs have been extensively discussed in several comprehensive reviews (Ribaut and Ragot 2007; Araus et al. 2008; Collins et al. 2008; Ribaut et al. 2009; Tuberosa and Salvi, 2009).

While genetic dissection of drought tolerance in maize seem to have been widely reported, successful accounts of practical application of identified QTLs in maize improvement programs have been scarce. The reasons are manifold, including genetic complexity, influence of genetic background, epistasis, profound QTL x environment interactions (QEI), population specific nature of identified QTLs, involvement of donor lines that are not agronomically elite among others (Collins et

al. 2008; Tsonev et al. 2009; Truntzler et al. 2010; Li et al. 2011). Integrating MAS in conventional breeding especially for drought related traits will be successful only when constitutive QTLs with effects of considerable size that express across a range of elite germplasm are identified. Meta-QTL analyses especially geared towards identification of genomic regions responsible for grain yield under water stress as well as well-watered conditions across a range of germplasm are a right step forward in this direction (Goffinet and Gerber 2000; Li et al. 2011; Swamy et al. 2011).

Single nucleotide polymorphisms (SNP) are the most common polymorphism among individuals of any species and have numerous advantages over all other marker systems such as high polymorphism, even distribution, co-dominance, high accuracy and reproducibility, high-throughput, rapid turn-around time and cost-efficiency (Xu et al. 2009; Deschamps and Campbell 2010). Most of the reported studies in maize till date have utilized SSRs for linkage map construction and QTL mapping. With the availability of whole genome sequence information in maize (Gore et al. 2009), most SNPs are physically anchored and provide an ideal platform for linkage mapping and QTL identification in maize, results of which could be easily compared with previous studies.

Considering the above outlined limitations of earlier mapping studies, we carried out QTL mapping using SNP markers in three tropical populations, involving elite lines across a wide range of environments under contrasting water regimes. Specifically, the objectives of the present investigation were to (1) identify genomic regions influencing GY and ASI across multiple environments under WS and WW conditions and estimation of their effect sizes; (2) determine the stability of the identified QTLs across diverse environments; (3) conduct a meta-analysis across three elite tropical populations to identify common genomic regions for GY and ASI and

(4) propose a set of SNP markers that physically delimit the identified meta-QTLs to enable integrating marker assisted selection for drought tolerance in the conventional maize improvement programs for the tropics.

Materials and methods

Plant materials

We evaluated three tropical maize populations that were developed by the Global Maize Program of CIMMYT. *Population 1:* CML444xMALAWI - consisted of 234 recombinant inbred lines (RILs), developed by single seed descent method. *Population 2*: CML440xCML504 - consisted of 247 $F_{2:3}$ families, obtained from randomly chosen F_2 plants. *Population 3*: CML444xCML441 – consisted of 300 $F_{2:3}$ families, obtained from randomly chosen F_2 plants. Inbred lines CML444, CML441, CML440 and CML504 were developed by CIMMYT, are adapted to mid-altitude (1000-1500 m asl) regions of sub-Saharan Africa and are considered to be tolerant to water limited conditions especially at flowering. Inbred MALAWI was developed in Zimbabwe and is considered to be relatively sensitive to water limited conditions, but has a high yield potential under optimal conditions. Inbreds CML444 and SC-Malawi are of late maturity (937 Male GDD), CML440 and CML441 mature early (824 and 870 Male GDD respectively) and CML504 is of early to intermediate maturity. Segregating families of all three populations CML444xMALAWI and CML444xCML441 were testcrossed to CML312, whereas families of CML440xCML504 were testcrossed to CML395 for phenotypic evaluations. Both testers are extremely sensitive to water scarcity.

Field experiments

The field experiments were conducted in Mexico (Tlatizapán station: 18ºN, 99ºW, 940m), Kenya (Kiboko station: 2º9'S, 37º75'E, 975m) and Zimbabwe (Harare station: 17°S, 31°E, 1468m and Chiredzi: 21ºS, 31ºE, 392m). Detailed characterization of these environments for drought phenotyping has been documented by Masuka et al. (2012). In Tlaltizapán, both well-watered (WW) and water stressed (WS) trials were conducted, whereas only WS trials were conducted in Kiboko. In Zimbabwe, the WW experiments were conducted in Harare and WS experiments were conducted at Chiredzi station. The soils at Tlaltizapán are classified as Vertisol, those at Kiboko are Arenosol, while the soils at Harare and Chriedzi are Alfisol. The trials were conducted in 2010 (both WW and WS) and 2011 (WS) in Mexico, whereas in Kenya and Zimbabwe the trials were conducted in 2010. In Zimbabwe the trials were planted in May at Chiredzi and Octuber at Harare. In Kenya trials were planted in June during the rain free period. Abbreviations for well watered environments were Mexico (MWW), Zimbabwe (ZWW) and water stress environments were Mexico in 2010 (MWS10), Mexico in 2011 (MWS11), Kenya (KWS) and Zimbabwe (ZWS).

The experimental design was alpha (0,1) lattice (Patterson and Williams 1976) with one-row plots and two replications at all the locations. In Mexico, plot size was of 5m and 0.75m between rows. Plots were planted with two seeds per hill and thinned to one plant per hill three weeks after planting resulting in a plant population of approximately 66,667 plants ha^{-1}. In Kenya, plot size was of 4m with 0.75m between rows and 20 cm between plants. Plots were planted with two seeds per hill and thinned to one plant per hill three weeks after planting resulting in a plant population of approximately 66,667 plants ha^{-1}. In Zimbabwe, the plots were 5 m long with spacing of 0.75 m between rows and 25 cm between plants in a row. Plots were

planted with two seeds per hill and thinned to one plant per hill three weeks after planting resulting in a plant population of approximately 53,333 plants ha^{-1}. Fertilizers, insecticides and herbicides were applied as required and in accordance with local recommendation practices. Drought stress was applied according to the established protocols in CIMMYT (Banziger et al. 2000), which is briefly described as below. In Mexico, water was applied to WS trials through furrow irrigation method at 10 days interval, until three weeks before the expected time of anthesis date (AD) in each population. This stress condition was maintained until five weeks after 50% of the families flowered. One more irrigation was applied during grain filling. The WS trials in Zimbabwe and Kenya were irrigated with sprinklers once a week until six and two weeks before and after flowering, respectively. In WW trials at all the locations, the soil moisture was maintained at about at field capacity.

The anthesis silking interval (ASI) was measured as the difference between the silking-date (SD) and anthesis-date (AD) in days (Bolaños and Edmeads 1996). AD is number of days from sowing to at least 50% of the pollen released per plot. SD is number of days from sowing to at least 50% silk emergence per plot. Mature ears were harvested manually, bagged, air-dried and shelled using an electric shelling device. The total grain yield of each plot was weighed on an electronic balance and converted to GY (t/ha) by dividing the total grain weight per plot by the plot area.

Statistical analyses of phenotypic data

Variance components were estimated from the standardized plot raw data by linear mixed model analysis using PROC MIXED of SAS (REML option). In all cases, AD was used as covariate. For individual analyses using alfa-lattice design and adjusting by a covariate, using the same syntaxes as in the SAS programs, the model is: $Y_{ijk} = \mu + \mathrm{Re}\, p_i + Block_j(\mathrm{Re}\, p_i) + Gen_k + Cov + \varepsilon_{ijk}$, where Y is the trait of interest,

μ is the mean effect, $\mathrm{Re}\, p_i$ is the effect of the i[th] replicate, $Block_j(\mathrm{Re}\, p_i)$ is the effect of the j[th] incomplete block within the i[th] replicate, Gen_k is the effect of the k[th] genotype, Cov is the effect of the covariate, and ε_{ijk} is the error associated with the i[th] replication, j[th] incomplete block, and k[th] genotype, which is assumed to be normally and independently distributed with mean zero and homocedastic variance σ^2.

For the combined analyses across locations by management or across all locations, the above models incorporate new terms. For the lattice design adjusted by a covariate, the model is:

$$Y_{ijkl} = \mu + Loc_i + \mathrm{Re}\, p_j(Loc_i) + Block_k(Loc_i\, \mathrm{Re}\, p_j) + Gen_l + (Loc_i \times Gen_l) + Cov + \varepsilon_{ijkl},$$

where the new terms Loc_i and $(Loc_i \times Gen_l)$ are the effects of the i[th] location and the location × genotype interaction, respectively.

When we are interested in calculating the BLUEs (LS means), both the genotypes and the covariate are considered fixed terms while all other terms are declared random terms; for calculating the broad-sense heritability, all terms are considered random, except the covariate. The broad-sense heritability was estimated by the formula:$h^2 = \sigma_G^2/(\sigma_G^2 + \sigma_{GE}^2/l + \sigma^2/lr)$, where σ_G^2 is the genotypic variance, σ_{GE}^2 is the genotype x environment interaction, σ^2 is the error variance, l is the number of environments and r is the number of replications in each trials.

For phenotypic and genotypic correlation the data was not adjusted by the covariate (AD). The phenotypic correlations among traits were calculated as simple Pearson's correlation coefficients based on adjusted and standardised phenotypic data. The genetic correlations among traits corresponded to the ratio between the genotypic covariance for each pair of traits and the product of the respective genotypic standard deviation.

Genetic maps

Genomic DNA was extracted from young leaves collected in a bulk of 15 plants per family/RIL and according to CIMMYT's Laboratory Protocols (CIMMYT, 2001). Genotyping was done with selected polymorphic markers for each population, from a set of 1536 SNPs (Yan et al. 2009). SNP genotyping was performed at Kbiosciences, UK using the KasPar chemistry.

Linkage maps in all the three populations were constructed using QTL IciMapping ver. 3.2 software (http://www.isbreeding.net) using the twin criterion of more than 3.0 LOD (Li et al. 2007). The recombination frequency between linked loci was transformed into centimorgan (cM) distances using Kosambi's mapping function (Kosambi 1944). In CML444xMALAWI, an integrated linkage map was constructed using 216 SNPs and 160 SSRs previously describe by Messmer et al. (2009; 2011), with which the length of the map was 2,349.23 cM. For the $F_{2:3}$ populations, CML440xCML504 and CML444xCML441, linkage maps were constructed using 194 and 265 SNPs, with 2,712.38 and 3,558.33 cM, respectively. The three distinct genetic maps were merged into a single integrated map using MetaQTL software version 1.0 (Veyrieras et al. 2007). The distances between adjacent markers from all individual maps were rescaled in Haldane unit. After integration of all the three maps, a consensus map of 620 markers was obtained. The consensus map had a total length of 1,484.45 cM with an average distance of 2.39 cM between markers (Fig. 1).

QTL analysis

Single and multiple environment QTL analysis

QTLs were identified for the adjusted means using Inclusive Composite Interval Mapping (ICIM) implemented in the software QTL IciMapping v.3.2 (Li et al. 2007).

Three procedures were realized to identify QTL in each population as follow: (1) mapping QTL for each individual environment, (2) mapping stable QTLs across combined WW and combined WS environments within each population and (3) mapping stable QTLs across all locations within each population. In all procedures the walking step in QTL scanning was 1 cM and likelihood odds (LOD) threshold of 2.5 was chosen for declaring the putative significant QTLs (Ribaut et al. 1997; Tuberosa et al. 2002). The stable QTLs were declared when the LOD of the QEI was below the threshold limit ($LOD_{QEI} \leq 2.5$). For $F_{2:3}$ populations, additive (a) and dominance (d) effects for each QTL as provided by QTL IciMapping v.3.2 were used to calculate the ratio of dominance level |d/a| and classified the nature of QTLs as per the criteria of Stuber et al. (1987), as follow: additive (A) = 0–0.20; partial dominance (PD) = 0.21–0.80; dominance (D) = 0.81–1.20, and overdominance (OD) > 1.20. The sign of the additive effects of each QTL was used to identify the origin of the favorable alleles as proposed by Lubberstedt et al. (1997).

QTL meta-analysis

QTL meta-analysis was performed with the MetaQTL software version 1.0 (Veyrieras et al. 2007). The statistical method implemented in this software hypothesizes that the input mapping studies are independent from each other. If redundant QTLs in the same population in different environments were detected, only QTLs with the highest effect (R^2) were kept in the analysis. The QTL intervals that were not supported by a minimum of two anchor markers were excluded from the meta-analysis. Also, repeated QTLs from the same population but detected in different environments were dropped. A meta-QTL was declared only when it was shared by all the three bi-parental populations. For a detailed explanation of the methods and procedures

adopted in meta-QTL analysis, see Danan et al. (2011).

Results

Phenotypic evaluations across different environments under two water regimes

The estimated means, genetic variance components, heritability and correlation between GY and ASI for the three populations are listed in Table 1. In general, drought stress significantly reduced the GY and increased the ASI across all the environments. In Mexico, between MWW and MWS10, the GY reductions were 41.3%, 28.8% and 47.2% in CML444xMALAWI, CML440xCML504 and CML444xCML441, respectively. In MWS11, the reduction in GY was more than 50% in all the three populations. The comparatively lower GY reduction in MWS10 was due to the unexpected rainfall in January (20.0 mm) and February (68.0 mm) of 2010. Across the three populations, combined GY means across all environments ranged from 1.91 to 9.23 t/ha, whereas GY under stress ranged from 0.1 to 6.76 t/ha. Across all the three populations, strong Genotype x Environment interaction (GEI) was observed while no significant negative correlation among locations was noted (Table S1) indicating wider adaptability of these populations across diverse environments.

Among the three populations, drought stress was most effective in CML444xCML441, which showed 63% GY reduction and 94% increase in ASI, based on combined water stress (WS) trials. Among the three populations, heritability for GY under combined WS (h^2_{GYws}) ranged from 0.31 to 0.46, while under combined WW environments (h^2_{GYww}), from 0.1 to 0.3. Strong GEI for WW locations (as indicated by lower h^2_{GYww}) and weak GEI (as indicated by higher h^2_{GYws}) in two populations (CML444xMALAWI and CML444xCML441) indicates the stability of

drought tolerant genotypes across diverse environments. CML444, which is the common parent shared by these two populations had been previously shown to be stable and high yielding under WS conditions (Messmer et al. 2009). Across the three populations, on an average, mean ASI increased by 3.3 days under WS conditions and mean ASI ranged between 0.12 to 11.2 days, while under WW conditions, from -2.0 to 7.0 days. The genetic variance of the ASI was higher in WS environments than in WW in all the three populations. Notably, the heritability of the combined analysis in WS environments (h^2_{ASIws}) was 0.5 and above, whereas h^2_{ASIww} in WW environments was 0.15 or below. This reinforces the earlier findings (Messmer et al. 2009, Lu et al. 2011) that reduced ASI is an important common drought adaptive mechanism among different drought tolerant genotypes. Significant and negative phenotypic (r_p) and genotypic (r_g) correlations between GY and ASI were observed across all WS environments across the three populations (Table 1). These correlations were mostly non-significant in WW environments. In some locations, it was not possible to estimate the r_g values because of the very low genetic variance for ASI, which were identified as NA^{ϕ} (Table 1).

Table 1. Estimates of means, genetic variance components, heritability and phenotypic (r_p) and genotypic (r_g) correlations between grains yield (GY) and anthesis-silking interval (ASI) for families on the three populations in single and multi-environments

	GY (ton/ha)								ASI (days)							Correlations	
Env.	Mean	Max	Min	σ_g^2	σ_{GEI}^2	σ^2	$\boldsymbol{h^2}$	CV	Mean	Max	Min	σ_g^2	σ_{GEI}^2	σ^2	$\boldsymbol{h^2}$	CV	r_p	r_g
CML444xMALAWI																		
MWW	9.60	14.69	4.43	1.41	-	2.68	**0.51**	17.64	0.74	5.31	-1.48	0.25	-	0.78	**0.39**	127.88	-0.06^{ns}	-0.14^{ns}
ZWW	7.19	12.33	3.10	0.56	-	3.85	**0.22**	30.13	0.91	13.50	-1.00	0.05^{ns}	-	0.67	**0.13**	91.01	-0.05^{ns}	-0.45**
MWS10	5.64	10.50	1.57	1.50	-	1.33	**0.74**	22.35	2.06	5.99	-2.47	0.65	-	2.29	**0.36**	74.23	-0.28**	-0.36**
MWS11	4.30	8.15	1.81	0.79	-	2.18	**0.63**	25.55	4.10	9.53	0.42	1.92	-	3.30	**0.54**	49.86	-0.51***	-0.87***
KWS	3.83	5.96	2.05	0.36	-	0.46	**0.61**	19.74	2.02	7.21	0.07	0.83	-	1.34	**0.55**	60.19	-0.40**	-0.67***
WW	8.58	11.60	5.54	0.14	0.56**	2.96	**0.10**	19.14	0.87	7.00	-0.75	0.07^{ns}	0.08^{ns}	1.57	**0.14**	118.11	-0.05^{ns}	NA$^{\phi}$
WS	4.64	6.76	2.20	0.21	0.91***	1.03	**0.31**	24.61	2.83	8.34	0.12	0.57	0.68***	2.34	**0.48**	45.67	-0.53**	NA$^{\phi}$
ALL	6.14	8.33	3.68	0.22	0.84***	1.95	**0.38**	15.99	2.03	5.11	-0.15	0.28	0.52***	2.05	**0.48**	44.27	-0.21**	NA$^{\phi}$
CML440xCML504																		
MWW	8.61	12.29	4.95	0.86	-	0.77	**0.69**	10.66	0.85	3.01	-0.50	0.12	-	0.73	**0.25**	104.06	-0.14*	-0.41*
ZWW	11.39	17.94	6.37	1.47	-	3.95	**0.43**	18.34	-0.06	2.50	-2.00	0.00^{ns}	-	1.33	**0.00**	-1946.11	-0.10^{ns}	-NA$^{\phi}$
MWS10	6.13	8.23	3.56	0.34	-	0.71	**0.49**	15.00	2.37	8.33	-0.32	0.39	-	1.77	**0.31**	59.67	-0.09^{ns}	-0.24*
MWS11	4.25	6.59	1.86	0.51	-	0.42	**0.71**	17.40	4.66	9.89	1.73	1.16	-	2.06	**0.53**	33.42	-0.53***	-0.86***
KWS	4.24	5.94	2.69	0.11	-	0.47	**0.32**	17.65	2.50	5.53	0.38	0.42	-	1.04	**0.45**	42.85	-0.28**	-0.66**
WW	10.00	14.22	6.88	0.61	0.55*	2.40	**0.41**	13.59	0.40	1.81	-0.79	0.01^{ns}	0.03^{ns}	1.14	**0.04**	195.24	-0.09*	NA$^{\phi}$
WS	4.88	6.36	3.38	0.11	0.22**	0.56	**0.39**	12.35	3.18	6.15	1.18	0.39	0.26***	1.63	**0.52**	27.47	-0.26**	-0.65**
ALL	6.93	9.23	5.00	0.23	0.46**	1.31	**0.51**	9.95	2.07	3.78	0.67	0.18	0.22***	1.41	**0.48**	30.34	-0.21*	-0.56**
CML444xCML441																		
MWW	10.81	14.15	2.93	1.73*	-	1.33	**0.72**	10.67	0.35	2.96	-2.03	0.17	-	0.38	**0.47**	186.81	-0.15*	-0.25*
ZWW	8.99	12.67	2.47	0.96*	-	1.75	**0.52**	14.71	0.33	2.00	-2.00	0.00^{ns}	-	0.33	**0.00**	339.80	0.15^{ns}	0.07^{ns}
MWS10	5.71	9.33	0.74	1.04*	-	0.84	**0.71**	17.56	3.07	7.32	0.45	0.92	-	1.20	**0.61**	38.17	-0.35***	-0.55***
MWS11	5.33	8.33	2.08	0.71*	-	0.68	**0.67**	17.55	4.70	9.04	0.37	1.14	-	1.74	**0.57**	30.89	-0.33***	-0.45**
KWS	1.83	3.40	0.09	0.02	-	0.42	**0.11**	37.15	6.85	15.81	1.51	2.00	-	7.23	**0.36**	42.73	-0.61**	NA$^{\phi}$
ZWS	1.81	3.40	0.39	0.06	-	0.48	**0.19**	39.59	8.00	16.25	0.66	0.24	-	12.43	**0.04**	45.27	-0.33***	NA$^{\phi}$
WW	9.91	12.66	3.27	0.38	1.05***	1.50	**0.30**	15.82	0.34	3.00	-2.00	0.09	0.00^{ns}	0.76	**0.32**	218.10	-0.21**	NA$^{\phi}$
WS	3.66	5.79	0.10	0.15	0.36***	0.63	**0.46**	18.25	5.65	11.22	1.48	0.72	0.37***	5.42	**0.48**	33.34	-0.39***	NA$^{\phi}$
ALL	5.73	7.53	1.91	0.30	0.49***	0.91	**0.66**	11.53	3.88	7.54	-0.29	0.44	0.33***	3.99	**0.53**	27.03	-0.67***	NA$^{\phi}$

***, ** and *significance at P <0.001, 0.01 and 0.05, respectively. WW: well-watered environments (Mexico and Zimbabwe), WS: water stress environments (Mexico10, Mexico11, Kenya and ZWS) and ALL: combined analysis across all environments.

QTL analysis

Single environment QTL analyses revealed 83 and 70 significant QTLs for GY and ASI, respectively, among the three populations (Table 2) with varying magnitude of effect sizes. In general, both parents in each of the three populations contributed positive alleles for both the traits. Most of the QTLs exhibited strong QEI, which was expected keeping in view the diverse environments across Latin America and Africa. In the RIL population of CML444xMALAWI, fewer number of QTLs were identified due to non-detection of dominant QTLs. QTLs detected in the two other $F_{2:3}$ populations across WS and WW environments predominantly showed partial to overdominant effects. In the population, CML440xCML504, around 30% of the QTLs for GY and 15% for ASI had additive effects (Table S2). Maximum number of QTLs was detected in CML444xCML441, in which however, only 10% of the QTLs had additive effects for GY and ASI (Table S4).

Table 2. Number of QTLs detected by three different mapping procedures in populations CML444xMALAWI, CML440xCML504 and CML444xCML441

Trait	Single environmental QTL						Joint per Management		Joint per All Env.
	MWW	ZWW	MWS10	MWS11	KWS	ZWS	WW	WS	WW+WS
GY	4/7/7	1/5/2	2/7/9	1/8/7	4/6/7	-/-/6	2/5/2	4/4/1	3/2/0
ASI	0/3/7	0/3/1	3/4/7	2/8/7	5/8/8	-/-/4	1/1/1	5/5/4	4/1/2

In each environment the / separated the population in the followed order (CML444xMALAWI/ CML440xCML504/ CML444xCML441). The symbol (–) indicates no information in a given environment.

CML444xMALAWI (Tolerant x Intermediary susceptible)

The single location, individual analyses revealed QTLs for GY on almost all the chromosomes, except on chr.3 and chr.6 (Table S2). In the combined analysis across WW environments, one QTL on chr.1 (at about 135.0 cM, 101.42-148.69 Mb) had large additive effects (0.62 t/ha in MWW and 0.31 t/ha in ZWW) and explained around 19% of the phenotypic variance (Table 3). This QTL also consistently showed up in the individual WW analyses (Table S2). The low R^2_{QEI} (2.1%) indicated the more stable nature of this QTL across WW environments. For ASI, we detected a minor QTL on chr.5 that explained around 3% of phenotypic variance across WW environments.

The combined analysis across WS environments revealed four significant QTLs for GY on chr.5, chr.7, chr.9 and chr.10. Interestingly, of the four QTLs, one on chr.10 was contributed by MALAWI, which is known to be more sensitive to drought stress (Messmer et al. 2009). QTLs on chr.5, chr.7 and chr.9 were also detected across ALL environment analysis, indicating their possible utility in selection decisions across WW and WS environments. Though none of these QTLs explained more than 6% of phenotypic variance for GY, most had very low R^2_{QEI} values indicating their stable nature across diverse environments. Viewed in conjunction with heritabilities for GY (0.31 in WS and 0.38 in ALL), these QTLs explain 7 to 18% of the genetic variance, which certainly merits their attention in marker based selection indices. The QTL on chr.5 was particularly interesting as it had relatively higher additive variance (R^2 = 4.1) as compared to R^2_{QEI} (0.63), which indicates its consistent performance across WS environments. In one of the WS environments (KWS), it explained close to 11% of phenotypic variance for GY (Table S2). In terms of additive genetic effects in WS environments, these QTLs had effects ranging from 0.01 to 0.33 t/ha. The QTL

on chr.7 had the highest additive genetic effect of 0.33 t/ha in MWS10. We detected five significant QTLs on chr.1, chr.2, chr.3 and chr.8 for ASI based on the combined WS environments, of which, the QTL on chr.3 (191.05 – 205.53 Mb) explained the largest (8%) phenotypic variance (Table 3) and also got detected in all individual WS environment analyses (Table S2). Contrary to QTLs for GY, most of the QTLs detected between WS and ALL environments for ASI were different.

CML440xCML504 (Tolerant x Tolerant)

The individual location QTL analyses for this population revealed 33 and 20 significant QTLs for GY and ASI, respectively, spread across all 10 maize chromosomes (Table S3). Unlike the CML444 x MALAWI (Tolerant x Intermediary susceptible) population, here, both the parents contributed with good number of QTLs influencing positively GY and ASI. Under WW conditions, both the parents contributed equally the favorable alleles at detected GY-QTLs, while CML440 contributed around 66% of favorable alleles for ASI QTLs. In WS environments, CML440 contributed 57% and 60% of favorable alleles at GY-QTLs and ASI-QTLs respectively. The phenotypic variance explained by individual location QTLs for GY ranged from 1.5 to 16% (Table S3).

In the combined analysis across WW environments, we detected five QTLs on chr.2, chr.4, chr.6, chr.8 and chr.9 for GY, which individually explained phenotypic variances ranging from 11 to 20%. As indicated by R^2_{QEI}, except one QTL on chr.9, all the others were consistent across WW environments. The QTL on chr.2 appeared very stable (R^2_{QEI} = 0.55) and explained around 20% of the phenotypic variance for GY, whose effects were 0.4 and 0.6 t/ha in MWW and ZWW respectively. The QTL on chr.9 exhibited the largest additive effect of 0.8t/ha in ZWW and the favorable

allele came from CML504. The other four GY-QTLs detected for WW environments were either partially dominant or over-dominant (Table 4). We detected four GY-QTLs in the combined analysis across three WS environments on chr.1, chr.5, chr.7 and chr.10, which individually explained phenotypic variances ranging from 7 to 17%. The predominant gene action was that of over-dominance for most QTLs detected under WS environments. The effect sizes in different WS environments for these GY-QTLs ranged from 0.02 to 0.18t/ha. The QTL on chr.7, which explained the largest phenotypic variance of 17% for GY in the combined WS analysis, was also detected in all the three individual WS environment analyses (Table S3). The QTL on chr.10 was also identified in two single WS environment analyses (Table S3). At the four GY-QTLs identified, CML504 contributed the favorable alleles for three of them (Table 3). The two QTLs on chr.2 and chr.6 identified in the WW analysis also showed up in the combined (ALL) analysis. We identified four QTLs for ASI based on combined analysis across the three WS environments on chr.2, chr.3, chr.5 and chr.9, which individually explained phenotypic variances varying from 8 to 15%. The ASI-QTL on chr.5 was the same as detected for GY in the combined WS analysis, corroborating the strong genotypic and phenotypic correlation between these two traits, especially under drought stress conditions. The QTL on chr.2 explained the largest phenotypic variance (15%) for ASI, performed consistently across the three WS environments (R^2_{QEI} = 1.9) with effects ranging from 0.14 to 0.36 days. The QTL on chr.9 had additive effects, while all the others exhibited mostly partially dominant or over-dominant mode of gene action (Table 3). No QTLs could be identified for ASI under WW or ALL environments.

CML444xCML441 (Tolerant x Tolerant)

We identified a total of 38 and 34 significant QTLs for GY and ASI, respectively based on individual environment QTL analyses (Table S4). The effect sizes of these GY-QTLs ranged from 0.10 to 0.98 t/ha in WW environments and 0.1 to 0.6 t/ha under WS environments. For ASI-QTLs identified in various individual environment analyses, the effects ranged from 0.1 to 1.5days. Favorable alleles at most of the large effect QTLs (R^2 = >10%) were contributed by CML444 for GY and CML441 for ASI with predominantly additive effects (Table S4). Based on the combined analysis across WW environments, we detected two QTLs on chr.2 and chr.3 for GY, of which, the QTL on chr.3 explained significant and large phenotypic variance of 25% and had effects of 0.9 and 0.1 t/ha in MWW and ZWW respectively (Table 5). Favorable alleles for both the QTLs were contributed by CML444. In the combined analysis across WS environments, only one QTL on chr.3 was identified which explained 10% of phenotypic variance with effects ranging from 0.07 to 0.15 t/ha. This QTL was also identified in all the four individual WS environment analyses (Table S4) with a wider range of effect sizes from 0.1 to 0.6 t/ha in different locations. Most of the identified QTLs exhibited over-dominant mode of gene action. No QTLs could be identified for GY in the combined (ALL) analysis (Table 5).

For ASI, one QTL was identified on chr.1 in the combined WW analysis, whereas four QTLs were detected in the combined WS analysis, indicating once again the importance of this trait under drought stress conditions. Conspicuously, CML441 contributed favorable alleles at all the identified ASI-QTLs, which highlighted the line's donor potential for introgressing this trait into other elite germplasm. The QTL on chr.2 explained the largest phenotypic variance of 17% with effects ranging from 0.1 to 0.5 days across various WS environments. The QTL on chr.3 appeared most

stable (as evidenced by lowest R^2_{QEI} of 1.9), with effects ranging from 0.1 to 0.5 days and explained close to 10% of the phenotypic variance. The delimited physical interval for this ASI-QTL was the same as that of GY-QTL detected on chr.3 under WS environments, which again explains the strong correlation between these two traits only under drought stress conditions. The ASI-QTLs identified on chr.6 and chr.7 were also detected in three out of four individual environment analyses, indicating their stable nature across diverse environments (Table 5, S4).

When we grouped all six environments in one combined analysis (ALL), no QTLs could be detected for GY, possibly due to huge GEI, indicating relatively narrower adaptation of this population. We identified two QTLs on chr.3 and chr.7 for ASI in the combined analysis (ALL), which together explained 18% of phenotypic variance, with effects ranging from 0.1 to 0.5 days (Table 5). While the QTL on chr.7 exhibited predominantly additive gene action, the one on chr.3 was over-dominant in three environments and dominant in another one. The QTL on chr.3 was identified consistently for GY as well as ASI across WS environments with significant effects and could be a potential target for marker assisted breeding programs.

Table 3. Genetic characteristics of QTLs for GY and ASI mapped across well-watered (WW), water stress (WS) and combined all five environments (ALL) on RILs in population CML444xMALAWI

	QTL Position								Additive Genetic Effects[3]					
					Physical	LOD									
Trait	**Env**[1]	**Chr**	**Pos. (cM)**	**Marker Interval**	**position**[2]	**G**	**QEI**	**R^2**	**R^2_{QEI}**	**MWW**	**ZWW**	**MWS10**	**MWS11**	**KWS**	**Direction**
GY	WW	1	135.0	pza02763.1-pza03200.2	101.42-148.69	7.35	0.01	18.93	2.11	0.62	0.31	-	-	-	CML444
	WS	5	225.0	bnlg1346-bnlg118	205.74-211.04	2.92	1.12	4.10	0.63	-	-	0.25	0.10	0.15	CML444
		7	82.0	phm9162.135-bnlg155	137.83-145.24	3.35	0.53	5.43	2.66	-	-	0.33	0.24	0.01	CML444
		9	85.0	umc81-pzb01899.1	49.03-98.50	3.00	0.00	3.49	0.32	-	-	0.17	0.20	0.09	CML444
		10	56.0	pza00337.4-bnlg1079	86.32-89.43	2.83	0.00	2.58	1.77	-	-	0.29	0.03	0.08	MALAWI
	ALL	5	228.0	bnlg118-phm3612.19	212.48-216.91	2.89	1.55	4.42	0.41	0.16	0.13	0.22	0.09	0.21	CML444
		7	82.0	phm9162.135-bnlg155	137.83-145.24	4.02	1.06	5.96	1.90	0.20	0.16	0.33	0.24	0.01	CML444
		9	89.0	umc81-pzb01899.1	49.03-98.50	2.97	0.00	3.19	0.19	0.13	0.11	0.17	0.17	0.09	CML444
ASI	WW	5	142.0	pzb01017.1-pza02641.2	158.03-168.92	2.64	0.00	3.19	0.00	0.10	0.09	-	-	-	MALAWI
	WS	1	139.0	pza02135.2-phm1809.18	166.55-183.29	2.61	0.00	1.62	0.66	-	-	0.11	0.26	0.05	CML444
		2	260.0	pzb00901.4-bnlg2042	9.40-9.54	2.74	0.14	3.08	0.04	-	-	0.22	0.17	0.18	CML444
		3	81.0	pza00210.9-zb21.1	29.69-37.53	2.89	0.00	4.92	0.95	-	-	0.14	0.39	0.19	CML444
		3	175.0	umc3b-umc16a	191.05-205.53	6.66	0.00	8.09	3.20	-	-	0.19	0.59	0.16	MALAWI
		8	55.0	phm2350.17-pza01301.1	23.98-82.23	4.64	0.00	7.03	2.98	-	-	0.05	0.51	0.30	MALAWI
	ALL	1	183.0	pza01039.1-phm3690.23	**-217.50	3.75	0.84	5.06	1.53	0.09	0.13	0.07	0.30	0.27	CML444
		3	137.0	bnl10.24a-PZA02212.1	170.95-174.55	2.53	1.46	4.01	1.68	0.12	0.00	0.24	0.12	0.28	MALAWI
		9	54.0	umc81-pzb01899.1	49.03-98.50	2.79	1.08	2.40	0.70	0.16	0.02	0.15	0.07	0.19	MALAWI
		10	62.0	pza00337.4-bnlg1079	86.32-89.43	2.72	0.00	2.26	0.58	0.03	0.06	0.17	0.16	0.15	CML444

[1]Joint analysis across WW: well-watered environments (MWW and ZWW), WS: water stress environments (MWS10, MWS11 and KWS) and combined all five environments (ALL). [2] Physical positions of the flanking markers on chromosomes in Mb (10^6 pb). LOD_G: LOD score for genetic effects across environments. LOD_{QEI}: LOD score of the QTL-by-environment interaction. R^2: percentage of phenotype variance explained by the QTL across environments. $R^2_{QEI:}$ phenotype variation explained by main effects QTL-by- environment interaction across environments. (**) unknown genomic region.

Table 4. Genetic characteristics of QTLs for GY and ASI mapped across well-watered (WW), water stress (WS) and combined all five environments (ALL) on $F_{2:3}$ population CML440xCML504

Trait	Env[1]	Chr	Pos (cM)	Marker Interval	Physical position[2]	LOD		R^2	R^2_{QEI}	Additive Effects[3]					Direction
						G	QEI			MWW	ZWW	MWS10	MWS11	KWS	
GY	WW	2	118.0	pza01755.1-pza01336.1	25.23-31.39	6.10	0.46	19.43	0.55	0.40^{PD}	0.59^{PD}	-	-	-	CML440
		4	250.0	phm5599.20-pza03322.5	239.24-242.02	2.52	0.50	11.97	0.44	0.25^{PD}	0.30^{PD}	-	-	-	CML504
		6	179.0	pzb01222.1-pza02815.25	164.41 - 167.88	3.32	2.51	13.77	1.38	0.10^{OD}	0.27^{OD}	-	-	-	CML440
		8	119.0	pza00739.1-pza01049.1	105.79-129.05	2.52	1.25	10.77	1.48	0.19^{OD}	0.21^{D}	-	-	-	CML440
		9	83.0	pza01791.2-pza000947.1	77.46 - 96.88	4.06	1.05	12.77	9.57	0.04^{OD}	0.79^{A}	-	-	-	CML504
	WS	1	262.0	pza03189.4-pza01267.3	64.26 - 76.05	2.85	2.32	8.40	4.95	-	-	0.18^{D}	0.05^{OD}	0.17^{PD}	CML504
		5	304.0	pza01680.3-pza02480.1	208.90-214.95	4.11	0.86	17.27	8.06	-	-	0.07^{OD}	0.05^{OD}	0.02^{OD}	CML440
		7	134.0	pza03166.1- pza02449.13	137.63-138.55	3.68	0.65	17.75	1.44	-	-	0.12^{OD}	0.12^{OD}	0.07^{OD}	CML504
		10	132.0	pza01141.1-phm3844.14	120.54-146.55	2.81	2.04	6.58	3.61	-	-	0.08^{D}	0.10^{PD}	0.20^{PD}	CML504
	ALL	2	118.0	pza01755.1-pza01336.1	25.23-31.39	8.45	1.38	20.35	13.92	0.37^{D}	0.59^{PD}	0.09^{PD}	0.22^{PD}	0.09^{PD}	CML440
		6	175.0	pzb01222.1-pza02815.25	164.41 - 167.88	6.55	9.57	16.92	6.08	0.15^{OD}	0.29^{OD}	0.14^{OD}	0.08^{OD}	0.03^{D}	CML440
ASI	WW	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	WS	2	111.0	pza01755.1-pza01336.1	25.23-31.39	5.34	2.05	14.77	1.98	-	-	0.32^{PD}	0.14^{OD}	0.36^{PD}	CML440
		3	139.0	phm2290.12-phm15449.10	121.88-125.08	2.72	0.88	8.41	2.93	-	-	0.09^{OD}	0.10^{OD}	0.14^{A}	CML504
		5	305.0	pza01680.3-pza02480.1	208.90-214.95	4.06	0.98	10.08	5.00	-	-	0.13^{A}	0.32^{D}	0.15^{PD}	CML504
		9	120.0	pza01096.1-phm4905.6	133.45-133.88	3.15	0.51	10.23	3.66	-	-	0.32^{A}	0.45^{A}	0.05^{D}	CML504
	ALL	--	--	--	--	--	--	--	--	--	--	--	--	--	--

[1]Joint analysis across WW: well-watered environments (MWW and ZWW), WS: water stress environments (MWS10, MWS11 and KWS) and combined all five environments (ALL). [2]Physical positions of the flanking markers on chromosomes in Mb (10^6 pb). LOD_G: LOD score for genetic effects across environments. LOD_{QEI}: LOD score of the QTL-by-environment interaction. R^2: percentage of phenotype variance explained by the QTL across environments. $R^2_{GEI:}$: phenotype variation explained by main effects QTL-by- The genetic action of the QTLs in each environment determined on the basis of the level of dominance: additive (A), partial dominance (PD), dominance (D) and overdominance (OD). (--) There are no stable QTLs for a give trait across environments.

Table 5. Genetic characteristics of QTLs for GY and ASI mapped across well-watered (WW), water stress (WS) and combined all six environments (ALL) on $F_{2:3}$ population CML444xCML441

Trait	Env[1]	Chr	Pos (cM)	Marker Interval	Physical position[2]	LOD		R^2	R^2_{QEI}	Additive Effects[3]						Direction
						G	QEI			MWW	ZWW	MWS10	MWS11	KWS	ZWS	
GY	WW	2	34.0	phm4586.12-pza01280.2	30.11-149.43	2.68	1.28	5.31	1.21	0.32^{A}	0.18^{D}	-	-	-	-	CML444
		3	231.0	pza00279.2-pza02616.1	52.80-210.16	11.19	2.38	24.57	11.48	0.85^{PD}	0.10^{D}	-	-	-	-	CML444
	WS	3	143.0	pza02098.2-pza03070.9	11.89-43.86	2.77	1.47	9.85	4.30	-	-	0.11^{OD}	0.15^{OD}	0.09^{OD}	0.07^{OD}	CML444
	ALL	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
ASI	WW	1	200.0	phm3034.3-pza01921.19	255.55-261.32	2.60	1.62	14.11	2.68	0.11^{PD}	0.11^{OD}	-	-	-	-	CML441
		2	125.0	pza01991.3-pza02727.1	220.40-227.92	3.86	0.57	16.65	4.66	-	-	0.13^{OD}	0.11^{OD}	0.51^{OD}	0.46^{D}	CML444
		3	140.0	pza02098.2-pza03070.9	11.89-43.86	2.57	0.52	9.55	1.98	-	-	0.07^{OD}	0.18^{OD}	0.32^{D}	0.46^{D}	CML441
	WS	6	133.0	phm12794.47-phm1190.3	120.23-128.48	4.37	2.11	14.77	4.70	-	-	0.13^{D}	0.36^{D}	0.43^{PD}	0.64^{D}	CML444
		7	80.0	pza01909.2-pza01210.1	6.44-75.10	2.81	0.28	5.93	2.05	-	-	0.09^{PD}	0.18^{A}	0.44^{A}	0.48^{A}	CML441
	ALL	3	140.0	pza00508.2-pza03070.9	11.89-43.87	3.18	1.18	9.89	4.11	0.09^{OD}	0.03^{OD}	0.07^{OD}	0.14^{OD}	0.29^{OD}	0.46^{D}	CML441
		7	82.0	pza01909.2-pza01210.1	6.44-75.10	3.70	1.36	7.56	4.65	0.16^{PD}	0.06^{D}	0.09^{D}	0.17^{A}	0.54^{A}	0.48^{A}	CML441

[1]Joint analysis across WW: well-watered environments (MWW and ZWW), WS: water stress environments (MWS10, MWS11, KWS and ZWS) and combined across all six environments. [2]Physical positions of the flanking markers on chromosomes in Mb (10^6 pb). LOD_G: LOD score for genetic effects across environments. LOD_{QEI}: LOD score of the QTL-by-environment interaction. R^2: percentage of phenotype variance explained by the QTL across environments. $R^2_{GEI:}$: phenotype variation explained by main effects QTL-by- The genetic action of the QTLs in each environment determined on the basis of the level of dominance: additive (A), partial dominance (PD), dominance (D) and overdominance (OD). (--) There are no stable QTLs for a give trait across environments.

Meta-analysis

Of the total 83 GY-QTLs identified by single environment analyses (Table 2), we plotted 56 on to a consensus map to perform a meta-QTL (mQTL) analysis, which enabled a larger overview of genomic regions across the three diverse bi-parental populations. We identified seven mQTLs for GY and one for ASI across the three populations with a confidence interval of 95% according described by Danan et al. 2011) (Table 6, Figure 1). Two GY-mQTLs each were identified on chr.1 and chr.10 and one each on chr.4, chr.5 and chr.7. The ASI-mQTL was found on chr.3. The confidence intervals for the seven mQTLs ranged from 2.39 to 12.63 cM, which was well below the arbitrary threshold of 30 cM as established by Hund et al. (2011). The sum of confidence intervals of plotted mQTLs covered only 3.2 % (46.93 cM) of the consensus map, built on the three populations. In Table 6, we also provide physical intervals for the identified mQTLs so to be able to compare them with the previously identified ones as well as for their utility in marker assisted breeding. The smallest delimited physical interval corresponded to 2.08Mb on chr.4 (mQTL_GY_4), flanked by pza03322.5 and pza01905.12. Except the mQTL on chr.5, which was delimited to a larger interval of 28 Mb, others were localized within narrower genomic regions, indicating the efficiency of the analysis. The mQTL on chr.1 (mQTL_GY_1a) had the largest number of QTLs integrated, which came from WW as well WS environments. While most of the mQTLs for GY included QTLs from both WW and WS environments, the mQTL on chr.7 (mQTL_GY_7) was solely based on five WS QTLs from all the three populations, which indicates that the region may play an important role in conferring adaptive drought response. As observed earlier, this region on chr.7 was consistently identified in CML444xMALAWI and CML440xCML504 across all the WS environments (Table 3 and 4) with low QEI. The favorable alleles at

mQTL_GY_7 were contributed by CML444, CML504 and CML441. The mQTL_GY_10 included three WS and two WW QTLs, which suggested its possible role in yield stability across both optimal and drought stress conditions. For ASI, only one mQTL was detected on chr.3 with a 8.48 Mb physical interval, which included 6 ASI-QTLs, all from WS environments, indicating the significance of this genomic region under drought stress conditions.

Using the annotated gene information available in maize database (www.maizesequence.org), candidate genes within the mQTL confidence intervals, with possible involvement in GY and ASI under WS and/or WW environments are presented in Table 7. These genes were involved in diverse networks controlling development, metabolism and responses to biotic and abiotic stresses.

Table 6. Meta-QTLs for GY and ASI across three maize subtropical bi-parental populations identified by meta-analysis

mQTL[1]	Chr	Pos. (cM)	Confidence interval (cM)	Flaking markers	Physical interval (Mb)	QTL number	QTL integrated[2]
mQTL_GY_1a	1	173.17	166.77-179.40	pza02741.1-phm1809.18	161.07-183.29 (22.22)	7	*pop1Gy1_MWW; pop1Gy1_ZWW; pop2Gy1_MWS10; pop2Gy1_KWS; pop3Gy1_ZWW; pop3Gy1a_MWS10; pop3Gy1a_MWS11*
mQTL_GY_1b	1	238.23	235.81-240.65	pza01588.1-pzd1403.1	275.98-285.27 (9.29)	4	*pop1Gy1_MWS11; pop2GY1b_KWS; pop3Gy1b_MWW; pop3_Gy1bMWS10*
mQTL_GY_4	4	99.15	97.95-100.34	pza03322.5-pza01905.12	242.02-244.10 (2.08)	5	*pop1Gy4_KWS; pop2Gy4_KWS; pop2Gy4_MWS11;pop2Gy4_MWW; pop3Gy4_MWS10*
mQTL_GY_5	5	132.33	129.70-134.97	pza00300.14-pza1142.2	171.69-199.70 (28.01)	5	*pop1Gy5_KWS; pop2Gy5_MWS10; pop3Gy5_MWS10; pop3Gy5_MWW; pop3Gy5_ZWS*
mQTL_GY_7	7	19.11	17.66-20.56	pza00986.1-bnl15.21	123.61-132.28 (8.67)	5	*pop1Gy7_MWS10; pop2Gy7_MWS10; pop2Gy7_KWS; pop2Gy7_MWS11; pop3Gy7_ZWS*
mQTL_GY_10a	10	36.48	33.56-39.41	pza00337.4-pza01292.2	86.32-109.63 (23.30)	5	*pop1Gy10_KWS; pop2Gy10_KWS; pop3Gy10_KWS; pop3Gy10_MWS11; pop3Gy10_MWW*
mQTL_GY_10b	10	49.95	46.75-53.18	pza03713.1-phm3736.11	121.49-147.76 (26.27)	5	*pop1Gy10_KWS; pop1Gy10_MWS10; pop2Gy10_MWS10; pop2Gy10_ZWW; pop3Gy10_MWW*
mQTL_ASI_3	3	99.36	96.05-102.67	pzd00027.2-pza01962.1	169.75-178.23 (8.48)	6	*pop1Asi3_KWS; pop1Asi3_MWS11; pop2Asi3_MWS10; pop3Asi3a_KWS; pop3Asi3b_KWS; pop3Asi3_ZWS*

[1] Meta-QTLs (GY for grain yield and ASI for anthesis-silking interval) followed by a chromosome number. [2] Detected QTLs by single QTL analysis in each population among different environments. The three populations were represented by the following order: pop1 (CML444xMalawi), pop2 (CML440xCML504) and pop3 (CML444xCML441). Mb= megabase (10^6 pb).

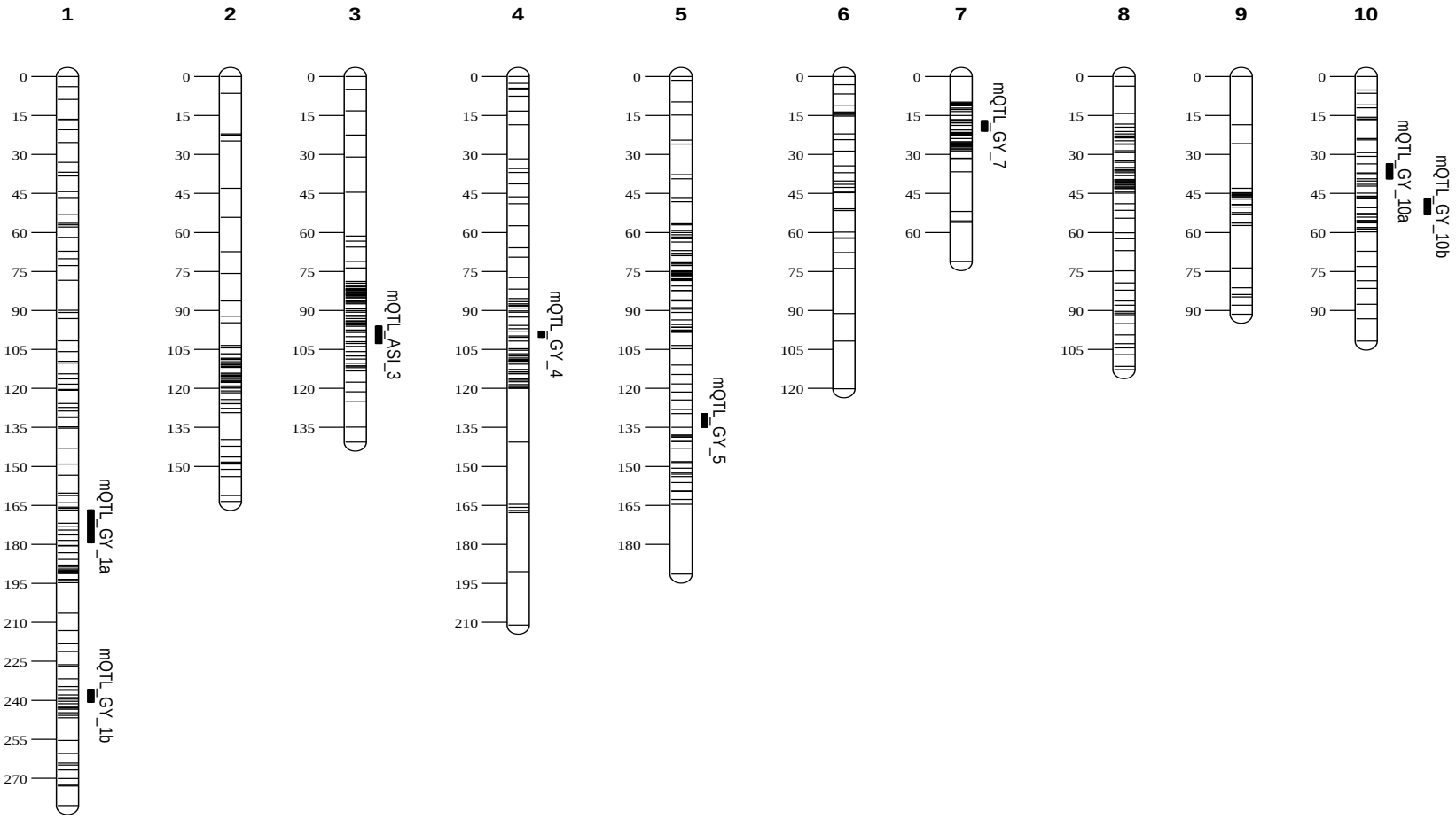

Fig. 1. Graphical overview of the grain yield (GY) and anthesis-silking interval (ASI) meta-QTLs in consensus map of the three maize populations. Ticks on the consensus chromosome indicate markers position and vertical tick bars to the right of chromosome represent meta-QTLs.

Table 7. Co-locating of the most important candidate genes related to drought tolerance in the physical intervals delimited by meta-QTLs

mQTL	Gene name	Gene position	Gene ID from Gramene	References[1]
mQTL_GY_1a	Cysteine synthase2(*cys2*)	177027403-177032403	GRMZM2G005887	Zhang et al. 2008
mQTL_GY_1b	exoglucanase1(*exg1*)	276305014-276310701	GRMZM2G147687	
	lethal embryo mutant1(*lem1*)	281107355-281109091	AC234157.1_FG002	
	phosphohexose isomerase1(*phi1*)	283086411-283088116	GRMZM2G065083	
	Aldehyde oxidade (*ZmAO3*)	285274032	GRMZM2G124260	Setter et al. 2011
mQTL_ASI_3	MADS-domain transcription factor (*Zmm16*)	171427820-171430412	GRMZM2G110153	Whipple et al. 2004, Dwivedi et al. 2008, Setter et al. 2011
mQTL_GY_5	petD	209941448-209965363	GRMZM2G427444	Raab et al. 2006
	glutathione transferase24(*gst24*)	211038250-211039523	GRMZM2G032856	Darkós et. 2011, Chen et al. 2012, Varga et al. 2012
mQTL_GY_7	glutathione transferase23(*gst23*)	128373591-128375197	GRMZM2G416632	Marrs et al. 1996, Darkós et. 2011, Chen et al. 2012, Varga et al. 2012
	isoamylase-type starch debranching(*iso3*)	129101506-129113096	GRMZM2G150796	
	glutathione S-transferase2(gst2)	90200517-90201913	GRMZM2G132093	Marrs et al. 1996; Darkós et. 2011, Chen et al. 2012, Varga et al. 2012
mQTL_GY_10a	cytochrome B6-F complex subunit 5 (*petG*)	90315936-90316307	GRMZM2G547408	Hu et al. 2010
	NADH dehydrogenase F(*ndhF*)	90140275-90144126	GRMZM2G405584	Casagrande et al. 2001, Rizhsky et al. 2004, Pastore et al. 2007
mQTL_GY_10b	lipoxygenase7(*lox7*)	120216863-120221081	GRMZM2G070092	Gigon et al. 2004, Peng et al. 2012
	glutamine synthetase1 (*gln1*)	146465615-146471079	GRMZM2G098290	Martin et al. 2006, Swarbreck et al. 2011, Yu et al. 2012
	transcription factor (*myb2*)	140048665-140050182	GRMZM2G081557	Cominelli et a. 2005, Dubos et al. 2010

[1]Studies reporting the active involvement of these genes with drought tolerance in maize or other species. Authors not listed in the reference section could be found in the supplementary reference.

Discussion

Ensuring an optimal drought stress is very critical to be able to detect QTLs with reasonable confidence intervals. Severe water stress conditions result in reduced genetic variance, thereby adversely affecting the chances of QTL detection (Ribaut et al. 1997). In this investigation, of the several WS environments, MWS10 experienced moderate drought stress and resulted in increased genetic variance for GY as compared to other WS and WW environments. Similar findings of reduced genetic variance and heritability estimates under severe water stress conditions were reported by Ribaut et al. (1997), Tuberosa et al. (2002), Messmer et al. (2009) and Lu et al. (2011). On the contrary, drought stress tends to increased genetic variance for ASI – the more severe the stress, the higher the ASI, which indicates the drought adaptive nature of this trait. In two of the three populations, the heritability estimates for GY under WW environments were considerably less than corresponding WS environments, indicating significant GEI, which is not surprising considering the diverse nature of environments. However, higher heritability estimates for GY under combined WS environments imply stability of drought tolerant genotypes across diverse environments.

Significant genotypic and phenotypic correlations were observed between ASI and GY, especially under WS environment, which has been previously well demonstrated in tropical maize germplasm (Bolaños and Edmeads 1996; Ribaut et al. 1997; Malosetti et al. 2008; Messmer et al. 2009; Lu et al. 2011). The strong correlation between GY and ASI is explained in part by the co-location of QTLs on chr.3 (11.9 to 43.9 Mb) and chr.5 (208.9 to 214.9 Mb) in populations CML440xCML504 and CML444xCML441, under WS environments. Besides, a number of co-locating QTLs were identified for GY and ASI on chr.1 and chr.10

based on single environment analyses. Similar overlapping genomic regions on chr. 1 and 10 for GY and ASI were also reported in QTL mapping experiments of Ribaut et al. (1997) and Malosetti et al. (2008), which explains stronger correlation of ASI with GY in a broad range of germplasm.

About 75% of the yield improvement in maize since 1930s has been attributed to genetic gain and the rest to agronomic practices (Araus et al. 2011). The substantial portion of this genetic gain was not associated with an increase in heterosis but rather with more stress tolerance (Duvick, 2005). Since the discovery of molecular markers, a number of QTLs influencing GY under stress and optimal water conditions in maize have been reported (Ribaut et al. 1996, 1997; Tuberosa et al. 2002; Lima et al. 2006; Guo et al. 2008; Malosetti et al. 2008; Messmer et al. 2009; Peng et al. 2011, Zhu et al. 2011), but information on QTLs that are stable across diverse environments and express more or less uniformly in different genetic backgrounds has been scarce (Li et al. 2010). Location and population-specific nature of QTLs coupled with inconsistent effect sizes has been a major bottleneck for their utilization in the breeding programs. Here, we report a set of constitutive and adaptive meta-QTLs for GY as well as ASI that were identified based on three biparental populations, derived from some of the elite tropical germplasm available at CIMMYT. Most of these meta-QTLs had moderate to reasonable effect sizes for GY and ASI across both WS and WW environments and were delimited to short physical intervals, thereby potentially enabling marker assisted breeding applications in future.

To the best of our knowledge, this is the first report of SNP markers based linkage map construction and QTL identification for drought related traits in tropical biparental maize populations. SNPs are the most common source of genetic variation and are the choicest marker systems owing to their amenability to automation and

high throughput applications. Among different crop species, maize is considered highly polymorphic with an average of 1% SNP frequency (Tenaillon et al. 2001) and more recently, Gore et al (2009) estimated based on first generation maize hapmap that one in every 44bp is polymorphic in maize genome. Besides, SNP markers in maize have been well-anchored to physical maps and provide opportunities to compare the identified genomic regions across a wide range of populations and germplasm as well as with previously reported SSR based studies.

In the present investigation, though different set of QTLs were identified across different water regimes, QTLs identified in a given WS or WW environment appeared stable across environments. In two of the three populations, average R^2_{QEI}, which is a measure of stability of a QTL, for WS QTLs was considerably less than average R^2_{QEI} for WW QTLs, indicating the stable nature of adaptive drought tolerant QTLs. These results are consistent with findings from other studies, which revealed that GY under WS and WW conditions is controlled by different set of genes (Ribaut et al. 1997; Lu et al. 2006; Messmer et al. 2009) and that a substantial proportion of QTLs detected under water stress regime did not present significant QEIs (Messmer et al. 2009).

We used an integrated map of SNPs and SSRs for CML444xMALAWI population and employed inclusive composite interval mapping (ICIM), which appeared to have improved the QTL detection power (Li et al. 2007). For instance, with the same population, Messmer et al. (2009) using 160 SSR markers and composite interval mapping detected QTLs for GY under both water regimes on chr.1, chr.5 and chr.8, of which however, nothing was common across the Mexican and African locations. Here, we were able to detect a QTL on chr.10 (86.32–89.43Mb) in CML444xMALAWI, which was expressed in both Mexico and Kenya

location, which possibly could be due to higher density of markers in the integrated map and the improved QTL detection methodology. Though a number of QTLs were identified in single environment QTL analyses in the current study as well as previous investigations, many of them were not stable across environments. However, a significant number of QTLs were commonly detected either in the same positions or over lapping physical intervals across three different populations, which prompted us to run meta-analysis using all the different QTLs identified in single environment analyses. We present here seven meta-QTL regions for GY and one for ASI that were identified based on their expression in all the three populations, either in one or more environments under two water regimes. Of the seven meta-QTLs for GY, except the one on chr.7, all other mQTLs integrated QTLs from WS as well as WW conditions. Particularly, mQTL_GY_1a and mQTL_GY_10b integrated almost equal number of QTLs under WS and WW environments, suggesting the constitutive nature of these genomic regions. The mQTL on chr.7 (mQTL_GY_7) solely integrated QTLs from WS environments, indicating the adaptive nature of this region. The rest of the regions were predominantly indicated by WS QTLs while integrating at least one WW QTL.

In the same physical interval as that of the constitutive mQTL_GY_1a (1.05/.06 at 161.07-183.29 Mb), a number of studies earlier have reported QTLs for GY and ASI, implying the significance of this region not only for WS conditions but also for optimal environments. Using RFLP markers in a F_3 population of tropical maize, Ribaut et al. (1997) identified a QTL on bin 1.06 for GY across WW and WS environments. Tuberosa et al. (2002) reported a SSR, *csu61b*, which is located between 180.71 to 181.19 Mb on chr.1 to be strongly linked with GY and root traits under both stress and optimal water conditions. More recently, Messmer et al (2009)

evaluating the recombinant inbred lines of CML444xMalawi identified a cluster of QTLs on bin 1.06 related to GY and other yield contributing traits under drought as well as well watered conditions in Mexican and African environments. A stable QTL for GY under WW conditions based on five Brazilian environments was detected in the physical interval of 91.46 to 185.02 Mb on chr.1 in yellow tropical maize germoplasm (Lima et al. 2006). Similarly, Lu et al. (2010) using a $F_{2:3}$ population, identified a QTL in 1.06 (164.55 to 195.05 Mb) for GY under WW conditions based on means across seven Asian environments. A recent meta-analysis involving 17 independent QTL mapping studies detected three strong genomic regions on chr.1, chr.7 and chr.10, of which the meta-QTL region on chr.1 was delimited to the physical interval, 178.87 to 180.72 Mb in 1.05/1.06 (Li et al. 2010), which together with Lima et al. (2006) and Lu et al. (2010) reinforces the constitutive nature of this genomic region.

Another region with strong evidence of being associated with constitutive response for GY was on chr.10 in the physical interval of 121.49 to 147.74 Mb (10.04 – 10.07), in which mQTL_GY_10b was located. Upstream of this region, another mQTL (mQTL_GY_10a) was identified at bin 10.04 about 86.33 to 109.63 Mb interval, which however was more prevalent in WS environments. The mQTL_GY_10b genomic region was also identified as stable QTL across WS environments in CML440xCML504, while mQTL_GY_10a was identified as stable in CML444xMALAWI. These regions are well corroborated by previous studies (Ribaut et al. 1997; Guo et al. 2008; Malosetti et al 2008; Hao et al. 2010; Li et al. 2010; Hao et al. 2011; Peng et al. 2011; Setter et al. 2011), which reported a number of GY and ASI QTLs under drought as well as WW conditions across diverse maize germplasm. In a meta-QTL analysis that integrated results from 12 QTL mapping

experiments, Hao et al. (2010) identified a significant genomic region for GY under WS conditions in bin 10.04 and another constitutive region for GY in bin 10.06. Another meta-QTL analysis by Li et al. (2010) involving seven populations that were not considered by the above study detected four important regions on chr. 10 for GY and related traits under drought conditions, all of which overlapped with the intervals delimited by the present investigation. Guo et al. (2008) reported a QTL for drought tolerance index on chr.10 at 141.86 to 146.06 Mb, whereas Peng et al. (2011) identified a QTL for GY under combined WW conditions at 111.84 to 126.62 Mb. Interestingly, using a mixed model approach, Malosetti et al. (2008) detected at 124.32 Mb, a strong QTL for drought as well as low nitrogen tolerance. Taken together, all these results imply that the genomic regions identified on chr.10 may play important roles in conferring yield advantages not only under drought stress but also in optimal environments. However, unless resolved through further fine mapping studies, it would be difficult to conclude whether many QTLs occur as a cluster in this region or pleiotropy of single genomic region is responsible for the manifold effects identified in the current and previous studies. Bioinformatic analysis of the physical interval (121.49 to 147.74 Mb) on chr.10 delimited to mQTL_GY_10b, based on the 'Named Genes' annotation track (http://www.plantgdb.org/ZmGDB) revealed the following three important candidate genes that have been previously linked to GY under drought and/or optimal conditions either in maize or other species - lipoxygenase7 (*lox7*) (GRMZM2G070092), glutamine synthetase1 (*gln1*) (GRMZM2G098290) and *Myb2* transcription factor (GRMZM2G081557) (Table 7). Lipoxygenases have been reported to respond to not only biotic stresses but also to certain abiotic conditions such as water deficit and wounding (Bell and Mullet, 1991). Glutamine synthetase (GS) acts at the center of nitrogen flow in plant nitrogen

metabolism and has been strongly implicated in maize grain production (Martin et al. 2006; Swarbreck et al. 2011) and was demonstrated to increase kernel number by 30% upon over-expression of GS1-3. Interestingly, Medici et al (2003) showed that GS activity is not affected by drought in maize hybrids that were subjected to severe water stress, which tempts us to speculate that *gln1* may be a candidate for one of the QTLs identified under WW conditions in this region. *Myb2* transcription factor has been shown to be an important transcriptional modulator of physiological responses in guard cells through a null mutation in *AtMyb60*, which resulted in the constitutive reduction of stomatal opening and in decreased wilting under water stress conditions (Cominelli et al 2005 and Dubos et al 2010).

Chromosome 1 harbored another mQTL genomic region downstream to the earlier described one, at 275.98 to 285.27 Mb, which predominantly integrated QTLs for GY under WS conditions and one for GY under WW environment. Using a $F_{2:3}$ population of Qi319xHuangzaosi and SSRs markers, Peng et al. (2011) identified a stable QTL in 258.88-292.98 Mb interval, based on across location WW environments. Unlike mQTL_GY_1a, this region has not been reported by many earlier studies that used biparental populations. However, utilizing association mapping approach and a set of 1229 SNPs in a panel of about 350 inbred lines from CIMMYT, which consisted of 5 parental lines used in the current study, Setter et al. (2011) detected a significant SNP marker (PZB01403.4) at 285.27 Mb, linked to the expression of abscisic acid (ABA) levels in silks seven days after flowering under water stress condition across two years in Mexico. The SNP was located within a gene (GRMZM2G124260) with aldehyde oxidase activity that is known to catalyze a wide range of reactions, including ABA synthesis (Ibdah et al. 2009). ABA is a fundamental component of the complex mechanisms that allow the plant to match the

water supply with the water demand. This hormone has been shown to affect many traits influencing the water balance of the plant through mechanisms of dehydration avoidance and dehydration tolerance (Tuberosa et al. 2005). Another genome-wide association study using a set of 95 inbred lines that are parents of most popular hybrids in China using 1536 SNP chip identified three SNPs on chr.10 viz., PZB02529.1 (86.32 Mb), PZB0111.8 (134.03 Mb) and PZA03607.2 (141.82 Mb) strongly associated with GY, ASI and drought tolerance index across different environments (Hao et al (2011). Setter et al. (2011), using a panel of inbred lines and SNPs as mentioned above, identified a region associated with accumulation of phasic acid in maize ears on chr.10 at 138.76 Mb. This SNP was located within an aquaporin gene (GRMZM2G125023) that is shown to be essential for regulation of water movement in cells (Devis et al. 2012).

Two other constitutive genomic regions detected as meta-QTLs in the present investigation were on chr.4 and chr.5. The mQTL_GY_4 appeared to be novel as no other previous reports identified this region, which integrated 4 QTLs from WS and one from WW environment. The mQTL on chr.5 (171.69 – 199.70Mb, bin 5.05/06/07) was well supported by a number of studies (Messmer et al. 2009; Hao et al. 2010; Li et al. 2010) that evaluated either lines or hybrids under drought and/or WW conditions. Particularly, it is worth mentioning that the meta-analysis conducted by both Hao et al. (2010) and Li et al (2010) identified a constitutive QTL on 5.06 and an adaptive region on 5.07, thereby providing strong evidence for this delimited physical interval.

Unlike other mQTLs for GY that integrated at least one QTL under WW condition, the mQTL on 7.03 at 122.62–132.28 Mb interval integrated only QTLs from WS environments and hence appeared adaptive in nature. This region was also

found to be stable across all WS environments in CML444xMALAWI as well as CML440xCML504. The adaptive nature of this region is strongly supported by the meta-analysis of Li et al. (2010) and the association study of Hao et al. (2011), which reported two significant markers at 122.62 Mb and 133.37 Mb on bin 7.03 associated with GY, ASI and drought tolerance index, only under WS conditions.

Physical intervals delimited to mQTLs on chr.5 and chr.7 harbored genes belonging to Glutathione S-transferases (GST) family viz., *gst24*, *gst23* and *gst2* (Table 7). In plants, Glutathione S-transferases are known to play significant regulatory roles and are induced by diverse environmental stimuli such as dehydration, senescence, and wounding with increased GST levels used to maintain cell redox homeostasis and protect organisms against oxidative stress. GSTs were proposed to afford protection under various stress conditions by detoxifying endogenous plant toxins that accumulate as a consequence of increased oxidative stress (Marrs, 1996). Recently, enhanced activity of GSTs under water stress conditions was reported to confer selective advantages to maize doubled haploid lines (Darko et al. 2011) as well as winter wheat (Varga et al. 2012). Using a knock-out mutant for a GST gene in *Arabidopsis,* Chen et al. (2012) demonstrated that GSTs play a pivotal negative regulatory role in conferring drought and salinity tolerance to the mutant plants as compared to wild ones.

Similar to GY-mQTL in bin 7.03, the mQTL_ASI_3 was detected only across WS environments, which is consistent with the individual QTL analysis results wherein ASI was found to be relevant only under drought conditions. The physical interval delimited to mQTL_ASI_3 (96.05 -102.67 Mb) contained a candidate gene, *Zmm16* (GRMZM2G110153 - MADS-domain transcription factor) that has been

clearly implicated in reproductive organ development (Whipple et al. 2004; Dwivedi et al. 2008; Setter et al. 2011).

Rainfed maize cultivation, mainly in the tropics is often exposed to extended periods of water limitation, both during vegetative as well as reproductive phase, which necessitates selections for stable GY especially under WS conditions. At the same time, efforts to impart drought tolerance should not result in compromised GY under optimal conditions, which requires identification of genotypes that equally perform well under WS and WW conditions. In the present study, we have identified several families within three tropical biparental populations that combine high GY under WW environments with good tolerance to WS conditions (Table S5), which could serve as an excellent source of initial source population for marker-assisted recurrent selection in the tropical breeding programs (Seeds of these superior families could be requested from CIMMYT, by contacting the corresponding author). Though the genetics of GY under WW and WS conditions differ considerably, this study has demonstrated that identification of genomic regions which confer selective advantages under WS, without compromising the optimal GY potential is possible. The eight meta-QTL regions identified in the present investigation merit attention for their further utilization in the marker-assisted selection as well as marker-assisted recurrent selection activities within pedigree breeding and population improvement programs.

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Supplementary information:

Table S1. Phenotypic (below diagonal) and genotypic (above diagonal) correlations of environments based on three subtropical maize populations for GY

Population CML444xMALAWI						
Envir.	MWW	ZWW	MWS10	MWS11	KWS	ZWS
MWW	1	0.14^{ns}	0.31**	0.32*	0.20^{ns}	-
ZWW	0.05^{ns}	1	0.30**	-0.24^{ns}	0.12^{ns}	-
MWS10	0.17*	0.10^{ns}	1	0.24^{ns}	0.30**	-
MWS11	0.19**	-0.09^{ns}	0.13^{ns}	1	0.41***	-
KWS	0.14^{ns}	0.05^{ns}	0.10^{ns}	0.27***	1	-
ZWS	-	-	-	-	-	-
Population CML440x CML504						
MWW	1	0.54***	0.39**	0.22^{ns}	0.42***	-
ZWW	0.29***	1	0.37**	0.02^{ns}	0.05^{ns}	-
MWS10	0.22**	0.17*	1	0.21^{ns}	0.16^{ns}	-
MWS11	0.15*	0.01^{ns}	0.13^{ns}	1	0.24^{ns}	-
KWS	0.20**	0.02^{ns}	0.06^{ns}	0.11^{ns}	1	-
ZWS	-	-	-	-	-	-
Population CML444xCML441						
MWW	1	0.27^{ns}	0.63***	0.57***	-0.03^{ns}	0.44**
ZWW	0.17**	1	0.25^{ns}	0.31**	0.15^{ns}	0.32**
MWS10	0.45***	0.16^{ns}	1	0.46***	-0.13^{ns}	0.37**
MWS11	0.40***	0.19**	0.32***	1	0.00^{ns}	0.28^{ns}
KWS	-0.02^{ns}	0.09^{ns}	-0.04^{ns}	0.00^{ns}	1	0.49***
ZWS	0.17**	0.11^{ns}	0.14^{ns}	0.11^{ns}	0.18**	1

Numbers followed by ***, **, * and ^{ns} indicate significance at $P < 0.001$, 0.01, 0.05 and no-significant effect, respectively. (-) environments removed from the analysis due to very low genetic variance under drought stress.

Table S2. QTLs and their genomic position, LOD values, genetic effects, gene action and phenotypic variation explained (R^2) for GY and ASI QTLs mapped in different environments using RILs families of CML444xMALAWI

Env.	QTL[1]	Chr	Pos (cM)	Marker Interval	Physical position[2]	LOD	R^2 (%)	Add[3]	Direction
MWW	*Gy1*	1	153.0	csu61b-bnlg1057	190.95-191.0	3.93	7.52	0.43	CML444
	Gy2	2	239.0	phm12979.9-pzao2337.4	9.03-15.51	3.19	6.43	-0.39	MALAWI
	Gy7	7	113.0	bnl14.07-pza00795.1	159.41-162.15	6.41	12.29	0.55	CML444
	Gy9	9	121.0	umc105a-umc11a	17.81-34.66	4.56	12.27	0.54	CML444
	Asi	-	-	-	-	-	-	-	-
ZWW	Gy1	1	130.00	bnlg2086-phm10621.29	82.34-101.42	3.17	11.04	0.52	CML444
	Asi	-	-	-	-	-	-	-	-
MWS10	*Gy7*	7	77.0	phm3078.12-phi082	128.47-131.39	2.57	6.59	0.38	CML444
	Gy10	10	47.0	phm15331.16-pza01597.1	10.43 - 61.60	4.36	17.57	-0.62	MALAWI
	Asi3	3	123.0	pza00186.4-pzad00027.2	165.80 - 169.75	2.88	9.31	0.41	CML444
	Asi4	4	95.0	umc156a-pza00453.2	143.10 -166.28	2.74	11.56	0.46	CML444
	Asi10	10	126.0	bnlg236-umc1038	140.96- 148.09	3.35	10.67	0.44	CML444
MWS11	Gy1	1	264.0	bnlg1720-d8.3	264.72-265.19	2.85	6.68	0.31	CML444
	Asi3	3	173.0	umc7-umc3b	165.99-196.07	4.9	10.34	-0.65	MALAWI
	Asi5	5	48.0	umc147a-pza02462.1	5.48-6.82	3.84	8.78	0.59	CML444
KWS	*Gy4*	4	101.0	pza00453.2-bnl2291	166.28-168.61	6.57	19.38	0.33	CML444
	Gy5	5	230.0	bnlg118-phm3612.19	211.04-212.48	3.55	10.98	0.25	CML444
	Gy8	8	140.0	pza01964.29-umc1384	166.98-168.36	6.16	18.49	0.33	CML444
	Gy10	10	32.2	npi285a-pza00048.1	5.45- 98.58	3.20	7.24	-0.20	MALAWI
	Asi1	1	283.0	phm13362.3-umc147b	275.98- 281.86	3.74	7.75	-0.34	MALAWI
	Asi2	2	83.0	pza01352.5-pza01991.3	220.39-226.45	6.12	15.18	-0.48	MALAWI
	Asi3	3	149.0	pza02212.1-phm17210.5	174.55-178.22	4.57	10.64	-0.40	MALAWI
	Asi4	4	77.0	pza03409.1-csu100	128.63-136.06	3.22	8.18	-0.35	MALAWI
	Asi10	10	115.0	bnlg236-pza00130.9	140.96-143.26	3.06	7.44	-0.33	MALAWI

[1]Name of QTLs (*Gy* for grain yield and *Asi* for anthesis-silking interval) followed by the chromosome number. [2]Physical position of flanking marker of the QTL in Mb (10^6 bp). [3]QTLs with additive effects with positive values were contributed by the parent CML444 and QTL with negative values are from parent MALAWI.

Table S3. QTLs and their genomic position, LOD values, genetic effects, gene action and phenotypic variation explained (R^2) for GY and ASI QTLs mapped in different environments using $F_{2:3}$ families from CML440xCML504

	QTL Position						Genetic Effect[3]			Gene Action[4]	
Env.	QTL[1]	Chr	Pos (cM)	Marker Interval	Physical positon[2]	LOD	R^2 (%)	Add	Dom	|d/a|	Nature	Direction
MWW	*Gy2*	2	121.0	pza01755.1-pza01336.1	25.23-31.39	3.60	7.77	0.40	-0.40	1.00	D	CML440
	Gy3	3	304.0	pza01688.3-phm2423.33	223.67 - 227.68	2.81	6.70	-0.35	-0.37	1.06	D	CML504
	Gy4	4	228.0	phm5599.20-pza03322.5	239.23 - 242.02	3.90	3.54	-0.27	-0.25	0.95	D	CML504
	Gy5	5	155.0	pza00996.1-pza01530.1	37.78 -37.79	3.67	7.49	0.46	0.12	0.27	A	CML440
	Gy6	6	172.0	pzb01222.1-pza02815.25	164.41 - 167.88	4.64	8.81	-0.31	-0.57	1.83	OD	CML504
	Gy8	8	123.0	pza00739.1-pza01049.1	105.79 - 129.04	2.61	6.21	0.25	-0.49	1.97	OD	CML440
	Gy9	9	50.0	zhd1.1-pza01999.3	22.04 - 23.21	4.08	7.00	0.45	-0.02	0.05	A	CML440
	Asi1	1	653.0	phm1438.34-pza03578.1	212.39 - 252.22	3.14	12.87	0.30	-0.40	1.34	D	CML440
	Asi2	2	124.0	pza01336.1-phm4880.179	31.39 - 103.49	2.70	9.12	-0.34	-0.12	0.46	PD	CML504
	Asi9	9	90.0	pza00947.1-pzb01899.1	96.89 - 98.51	3.72	6.44	0.32	-0.16	0.49	PD	CML440
ZWW	*Gy2*	2	118.0	pza01755.1-pza01336.1	25.23-31.39	3.04	5.07	0.56	-0.32	0.57	PD	CML440
	Gy6	6	173.0	pzb01222.1-pza02815.25	164.41 - 167.88	3.78	5.34	0.37	-0.69	1.86	OD	CML440
	Gy8	8	137.0	pza00118.5-pza01049.1	126.15 - 129.04	2.66	5.42	0.62	-0.08	0.13	A	CML440
	Gy9	9	84.0	pza00947.1-pzb01899.1	96.88 - 98.50	5.01	9.32	-0.81	0.16	0.19	A	CML504
	Gy10	10	139.0	pza01141.1-phm3844.14	120.54 - 146.55	3.23	9.90	-0.39	-1.05	2.66	OD	CML504
	Asi1a	1	136.0	pza03521.1-pza00887.1	10.068 - 10.934	2.58	4.94	-0.20	0.23	1.15	OD	CML504
	Asi7	7	163.0	pza01542.1-pza02449.13	129.79 - 134.84	3.72	10.31	0.09	0.51	5.63	OD	CML440
MWS10	*Gy1*	1	260.0	pza03189.4-pza01267.3	64.26 - 76.05	3.79	10.37	0.10	-0.50	4.85	OD	CML440
	Gy4	4	160.0	pza02779.1-phm1684.20	207.11 - 209.04	2.68	1.42	0.04	0.18	4.34	OD	CML440
	Gy5	5	178.0	pza01530.1-pza02408.2	37.79 - 189.41	2.95	2.39	-0.17	-0.07	0.40	PD	CML504
	Gy6	6	172.0	pzb01222.1-pza02815.25	164.41 - 167.88	4.75	8.83	0.18	-0.41	2.27	OD	CML440
	Gy7	7	164.0	pza01542.1-pza02449.13	129.79-138.55	2.82	1.41	-0.05	-0.19	3.8	OD	CML504
	Gy8	8	211.0	phm1834.47-phm4560.54	162.44 - 163.53	2.84	5.90	0.05	-0.39	8.28	OD	CML440
	Gy10	10	137.0	pza01141.1-phm3844.14	120.53 - 146.55	2.86	9.56	-0.14	0.44	3.14	OD	CML504
	Asi1	1	138.0	pza00887.1-pza03521.1	10.07 - 10.93	4.45	7.73	0.41	-0.34	0.83	D	CML440
	Asi2	2	97.0	pza00590.1-pza01755.1	21.99 - 25.23	3.21	4.87	0.37	-0.13	0.35	PD	CML440
	Asi3	3	148.0	phm2290.12-phm15449.10	121.88 - 125.23	2.94	3.26	-0.10	0.37	3.7	OD	CML504
	Asi5	5	65.0	pza01570.1-pza03092.7	3.53 - 11.99	2.97	6.99	0.26	-0.47	1.84	OD	CML440

MWS11	*Gy2a*	2	34.0	phm4425.25-phm6111.5	19.84-21.99	5.56	8.13	0.41	0.02	0.04	A	CML440
	Gy2b	2	111.0	pza01755.1-pza01336.1	25.23-31.39	2.76	4.80	0.26	-0.15	0.57	PD	CML440
	Gy3	3	209.0	pza01962.12-pza03458.1	178.23-203.32	3.22	5.14	0.31	-0.06	0.21	A	CML440
	Gy4	4	229.0	phm5599.20-pza03322.5	239.24-242.02	4.25	4.07	0.22	-0.17	0.79	PD	CML440
	Gy6	6	170.0	pzb01222.1-pza02815.25	164.41 - 167.88	2.55	2.53	0.12	-0.23	1.91	OD	CML440
	Gy7	7	164.0	pza01542.1-pza02449.13	129.79-138.55	2.91	1.52	-0.01	-0.24	24.0	OD	CML504
	Gy8	8	143.0	pza00118.5-phm4203.11	126.16-133.53	3.27	5.59	-0.16	-0.43	2.71	OD	CML504
	Gy9	9	150.0	pza02235.14-pza00708.3	132.12-147.38	4.81	13.76	0.45	-0.19	0.41	PD	CML440
	Asi2	2	69.0	phm6111.5-pza00590.1	21.99-29.99	4.05	4.28	0.15	-0.59	3.94	OD	CML440
	Asi4	4	57.0	pza03385.1-phm14717.2	37.07-40.52	3.16	3.61	-0.36	-0.24	0.66	PD	CML504
	Asi5a	5	242.0	pza00963.3-phm3512.186	203.43-207.27	3.33	4.46	-0.14	0.58	4.31	OD	CML504
	Asi5b	5	308.0	pza01680.3-pza02480.1	208.90-214.95	4.15	8.14	-0.35	-0.66	1.88	OD	CML504
	Asi7	7	164.0	pza01542.1-pza02449.13	129.79-134.85	3.63	4.48	0.15	0.64	4.15	OD	CML440
	Asi8	8	74.0	pza01079.1-phm2350.17	14.12-23.99	5.15	7.18	0.64	-0.10	0.16	A	CML440
	Asi9a	9	51.0	zhd1.1-pza01999.3	22.04-23.22	7.13	9.14	0.08	-0.90	10.64	OD	CML440
	Asi9b	9	118.0	pza02397.1-phm4905.6	133.92-133.92	5.28	6.59	-0.63	0.06	0.10	A	CML504
KWS	*Gy1*	1	260.0	pza03189.4-pza01267.3	64.26 - 76.05	3.15	6.21	-0.21	0.03	0.15	A	CML504
	Gy1	1	465.0	pza00343.31-phm4752.14	294.64 - 298.87	5.74	12.12	0.04	-0.42	10.65	OD	CML440
	Gy4	4	71.0	pza02289.2-pza00941.2	180.31 - 185.56	3.92	5.83	-0.21	-0.02	0.08	A	CML504
	Gy7	7	164.0	pza01542.1-pza02449.13	129.79-138.55	3.06	1.57	-0.04	0.17	4.25	OD	CLM504
	Gy8	8	191.0	phm4757.14-phm1834.47	151.45 - 162.44	5.35	10.96	0.03	0.40	12.28	OD	CML440
	Gy10	10	140.0	pza01141.1-phm3844.14	120.53 - 146.55	6.51	15.86	-0.27	0.25	0.75	D	CML504
	Asi1	1	661.0	phm1438.34-pza03578.1	212.39 - **	4.16	11.14	-0.49	0.13	0.26	PD	CML504
	Asi8	8	52.0	phm2487.6-pza01079.1	8.23 - 14.12	4.23	7.86	0.09	-0.52	5.61	OD	CML440
	Asi9	9	9.0	pza01386.3-phm5181.10	12.21 - 15.58	2.92	4.10	0.25	-0.23	0.94	D	CML440

[1]Name of QTLs (*Gy* for grain yield and *Asi* for anthesis-silking interval) followed by a chromosome number. [2] Physical position of flanking markers of the QTL in Mb (10^6 bp). [3]Predominant genetic effect of a QTL is indicated by A: additive and D: dominant. QTLs with additive effect with positive values were contributed by the parent CML440 and QTL with negative values are from parent CML504. [4]Gene action determined on the basis of the level of dominance calculated by the ratio between dominant and additive effects of the QTLs (|d/a|) using Stuber et al. (1987) criterion: additive (A) = 0 – 0.20; partial dominance (PD) = 0.21 – 0.80; dominance (D) = 0.81 – 1.20, and overdominance OD > 1.20. ** unknown physical position.

Table S4. QTLs and their genomic position, LOD values, genetic effects, gene action and phenotypic variation explained (R^2) for GY and ASI QTLs mapped in different environments using $F_{2:3}$ families from CML444xCML441

	QTL Position						Genetic Effect[3]				Gene Action[4]	
Env	QTL[1]	Chr	Pos (cM)	Marker Interval	Physical positon[2]	LOD	R^2 (%)	Add	Dom	|d/a|	Nature	Direction
MWW	*Gy1a*	1	144.0	pza03578.1-d8.2	252.22-265.20	2.93	1.47	-0.24	0.08	0.34	PD	CML441
	Gy1b	1	487.0	pza03183.5-pza03189.4	46.07-64.26	2.66	2.09	0.10	-0.46	4.62	OD	CML444
	Gy2	2	62.0	pza01280.2-phm3668.12	149.43-195.56	2.78	3.22	0.33	-0.32	0.97	D	CML444
	Gy3	3	227.0	pza00279.2-pza00279.2	52.80-210.16	13.27	23.69	0.98	0.48	0.49	PD	CML444
	Gy5	5	363.0	phm5798.39-pza01304.1	71.10-178.58	3.40	1.63	-0.21	0.15	0.74	PD	CML441
	Gy6	6	50.0	pza00355.2-phm2551.31	78.76-85.13	2.51	3.19	0.22	-0.47	2.13	OD	CML444
	Gy10	10	318.0	phm15868.56-pza02527.2	137.13-148.49	3.00	6.56	0.52	0.41	0.78	PD	CML444
	Asi1a	1	145.0	pza03578.1-d8.2	252.22-265.19	2.57	1.85	-0.10	0.18	1.80	OD	CML441
	Asi1b	1	571.0	phm595.30-pza02087.2	281.82-284.06	3.28	4.94	-0.15	-0.16	1.05	D	CML441
	Asi3	3	114.0	phm2423.33-pza00297.2	39.99-227.68	4.62	7.95	-0.21	-0.29	1.38	OD	CML441
	Asi4	4	280.0	pza02027.1-pza03459.1	132.98-134.29	2.66	6.27	-0.15	-0.32	2.14	OD	CML441
	Asi5	5	165.0	pza00963.3-pza02015.11	207.27-207.46	2.87	4.33	-0.19	0.00	0.01	A	CML441
	Asi7	7	63.0	pza01909.2-pza01210.1	6.44-75.09	3.38	3.30	-0.15	0.17	1.13	D	CML441
	Asi10	10	96.0	pza01001.2-phm3736.11	146.54-147.76	3.38	9.20	-0.26	-0.19	0.73	PD	CML441
ZWW	*Gy1*	1	306.0	phm5622.21pza02467.10	183.83-196.93	4.02	12.96	0.63	0.20	0.31	PD	CML444
	Gy2	2	234.0	pza00365.2-pza02337.4	1.22-15.51	2.92	7.96	0.37	0.70	1.90	OD	CML444
	Asi1	1	195.0	phm3034.3-pza01921.19	255.55-261.32	3.05	11.49	-0.28	-0.48	1.70	OD	CML441
MWS10	*Gy1a*	1	324.0	pza03200.2-phm5622.21	148.69-183.83	6.18	9.51	0.50	0.10	0.20	A	CML444
	Gy1b	1	486.0	pza03183.5-pza03189.4	46.06-64.26	2.81	2.66	0.12	-0.41	3.43	OD	CML444
	Gy2	2	164.0	pza02264.5-pzb00901.4	3.17-9.41	3.89	5.71	0.43	-0.35	0.81	PD	CML444
	Gy3a	3	117.0	pza00297.2-pza03070.9	39.99-43.86	2.93	1.21	0.00	0.27	∞	OD	CML444
	Gy3b	3	278.0	pza01154.1-phm2672.19	216.03-219.86	3.69	4.95	0.34	-0.38	1.11	D	CML444
	Gy4	4	85.0	phm4117.14-phm5780.13	215.39-237.58	8.27	12.62	0.58	0.14	0.24	PD	CML444
	Gy5a	5	62.0	pza02480.1-pza02769.1	214.95-215.51	3.15	3.53	0.17	0.35	2.04	OD	CML444
	Gy5b	5	363.0	phm5798.39-pza01304.1	71.10-178.58	3.13	1.92	0.00	0.38	∞	OD	CML444
	Gy10	10	270.0	phm5740.9-pzb01301.5	8.77-9.75	3.77	4.72	-0.17	0.42	2.49	OD	CML441
	Asi1	1	573.0	phm595.30-pza02087.2	281.82-284.06	5.10	8.10	-0.49	-0.10	0.20	A	CML441
	Asi2	2	262.0	pza01232.1-pza02939.10	155.87-157.15	6.10	10.13	-0.50	0.16	0.33	PD	CML441
	Asi3	3	114.0	phm2423.33-pza00297.2	39.99-227.68	3.08	2.08	-0.23	-0.27	1.17	D	CML441

	Asi5	5	370.0	pza02207.1-pza01304.1	49.20-178.58	3.40	3.42	-0.13	-0.51	3.99	OD	CML441
	Asi6	6	98.0	pza00214.1-phm12794.47	91.70-128.48	2.53	3.72	0.22	-0.46	2.09	OD	CML444
	Asi10a	10	150.0	pza01456.2-phm3844.14	135.93-146.55	3.22	6.82	0.06	-0.66	11.72	OD	CML444
	Asi10b	10	318.0	phm15868.56-pza02527.2	137.13-148.49	3.29	5.39	-0.28	-0.60	2.13	OD	CML441
MWS11	*Gy1a*	1	44.0	pzb01227.6-pza00623.3	288.44-293.63	2.92	2.46	0.18	0.19	1.05	D	CML444
	Gy1b	1	338.0	pza03200.2-pza02741.1	148.64-161.07	2.95	3.41	0.28	0.00	0.00	A	CML444
	Gy2	2	67.0	pza01280.2-phm3668.12	149.43-195.56	2.73	3.58	0.13	-0.37	2.83	OD	CML444
	Gy3a	3	113.0	pza00297.2-pza03070.9	39.99-43.86	6.07	10.32	0.11	0.73	6.97	OD	CML444
	Gy3b	3	221.0	pza00279.2-pza02616.1	52.80-210.16	11.02	19.02	0.58	0.31	0.54	PD	CML444
	Gy5	5	63.0	pza02480.1-pza02769.1	214.95-215.51	3.13	3.26	0.28	-0.03	0.10	A	CML444
	Gy10	10	271.0	phm5740.9-pzb01301.5	8.77-9.75	2.86	2.55	-0.24	0.04	0.34	PD	CML441
	Asi1a	1	48.0	pzb01227.6-pza00623.3	288.44-293.63	2.85	2.45	0.18	0.20	1.13	D	CML441
	Asi1b	1	491.0	pza03183.5-pza03189.4	46.06-64.26	3.01	3.34	0.14	0.45	3.09	OD	CML444
	Asi3a	3	116.0	phm2423.33-pza00297.2	39.99-227.68	2.88	0.97	-0.19	0.20	1.05	D	CML441
	Asi3b	3	274.0	pza01154.1-phm2672.19	216.03-219.86	2.56	2.55	-0.12	0.48	4.04	OD	CML441
	Asi6	6	98.0	pza00214.1-phm12794.47	91.70-128.48	2.72	2.85	0.37	-0.11	0.30	PD	CML444
	Asi7	7	62.0	pza01909.2-pza01210.1	6.44-75.09	2.65	0.98	-0.24	0.06	0.20	A	CML441
	Asi10	10	100.0	pza01001.2-phm3736.11	146.54-147.76	2.55	3.60	-0.33	-0.38	1.15	D	CML441
							14.79					
KWS	*Gy1a*	1	47.0	pzb01227.6-pza00623.3	288.44-293.63	3.19	7.17	0.05	-0.27	5.02	OD	CML444
	Gy1b	1	397.0	pzb00872.3-pzb01062.3	46.25-56.85	4.02	6.72	0.18	0.07	0.37	PD	CML444
	Gy2	2	138.0	pza02727.1-phm482.27	11.10-227.92	3.11	5.73	-0.49	0.47	0.97	D	CML441
	Gy3a	3	115.0	pza00297.2-pza03070.9	39.99-43.86	2.86	2.16	0.00	-0.41	∞	OD	CML441
	Gy4	4	302.0	fea2.3-pza02194.1	132.74-180.31	2.88	4.21	0.15	0.06	0.37	PD	CML444
	Gy9	9	38.0	pzb01110.6-pza01062.1	88.06-24.03	5.57	12.90	0.27	-0.01	0.03	A	CML444
	Gy10	10	319.0	phm15868.56-pza02527.2	137.13-148.49	3.43	6.49	-0.02	-0.28	15.70	OD	CML441
	Asi1	1	398.0	pzb00872.3-pzb01062.3	46.26-56.85	4.95	7.21	-0.93	-0.26	0.28	PD	CML441
	Asi2	2	134.0	phm482.27-pza02727.1	11.10-227.92	4.33	6.45	0.98	-0.48	0.50	PD	CML444
	Asi3a	3	118.0	pza00297.2-pza03070.9	39.99-43.86	3.46	3.09	-0.38	0.90	2.38	OD	CML441
	Asi3b	3	249.0	pza02516.1-pza03391.1	219.86-208.18	3.20	4.74	-0.25	-0.93	3.70	OD	CML441
	Asi7	7	69.0	pza01909.2-pza01210.1	6.44-75.09	3.48	4.28	-0.76	0.04	0.05	A	CML441
	Asi9a	9	37.0	pzb01110.6-pza01062.1	24.03-88.06	2.67	3.06	-0.50	0.39	0.78	PD	CML441
	Asi9b	9	177.0	phm816.29-pza01715.2	142.05-142.95	4.63	6.76	-0.53	-0.88	1.65	OD	CML441
	Asi10	10	267.0	phm5740.9-pzb01301.5	8.77-9.75	3.32	4.35	-0.75	-0.19	0.25	PD	CML441

ZWS	*Gy1a*	1	217.0	pza01921.19-pza03064.9	261.31-294.41	2.61	5.77	0.14	0.16	1.14	D	CML444
	Gy1b	1	527.0	pza02284.1-phm1653.32	9.27-14.89	4.60	10.22	0.01	0.36	36.0	OD	CML444
	Gy2	2	234.0	pza00365.2-pza02337.4	1.22-15.51	2.51	4.70	0.12	0.22	1.91	OD	CML44
	Gy3	3	119.0	pza00297.2-pza03070.9	39.99-43.86	3.38	5.25	0.03	-0.24	6.91	OD	CML444
	Gy5	5	367.0	pza01304.1-pza02207.1	49.20-178.58	3.17	5.25	0.15	0.17	1.13	D	CML444
	Gy7	7	108.0	pza00153.7-pza01946.7	**-123.60	3.47	9.07	-0.22	0.20	0.93	PD	CML441
	Asi1	1	116.0	d8.2-pzb00114.1	265.20-275.98	2.53	4.45	-0.76	-0.99	1.30	OD	CML441
	Asi3	3	120.0	pza00297.2-pza03070.9	39.99-43.86	2.78	3.02	-0.62	-0.59	0.95	D	CML441
	Asi6	6	127.0	phm1190.3-phm12794.47	120.23-128.48	3.26	7.33	0.75	0.83	1.10	D	CML444
	Asi7	7	61.0	pza01909.2-pza01210.1	6.43-75.09	3.85	12.27	-1.48	0.80	0.54	PD	CML441

[1]Name of QTLs (*Gy* for grain yield and *Asi* for anthesis-silking interval) followed by a chromosome number. [2] Physical position of flanking markers of the QTL in Mb (10^6 bp). [3]Predominant genetic effect of a QTL is indicated by A: additive and D: dominant. QTLs with additive effect with positive values were contributed by the parent CML444 and QTL with negative values are from parent CML441. [4]Gene action determined on the basis of the level of dominance calculated by the ratio between dominant and additive effects of the QTLs (|d/a|) using Stuber et al. (1987) criterion: additive (A) = 0 – 0.20; partial dominance (PD) = 0.21 – 0.80; dominance (D) = 0.81 – 1.20, and overdominance OD > 1.20. ** unknown physical position.

Table S5. Means of the best and worst families under water stress (WS) and their corresponding values under well watered (WW) environments in three bi-parental maize populations.

	Best families			Worst families		
Population	Pedigree	WS	WW	Pedigree	WS	WW
CML444xMALAWI	CML444/MALAWI-291	6.76	8.10	CML444/MALAWI-395	2.98	6.95
	CML444/MALAWI-330	6.49	8.81	CML444/MALAWI-205	2.89	10.81
	CML444/MALAWI-352	6.42	11.22	CML444/MALAWI-120	2.80	10.39
	CML444/MALAWI-52	6.41	10.74	CML444/MALAWI-230	2.78	8.77
	CML444/MALAWI-49	6.31	10.02	CML444/MALAWI-322	2.74	10.46
	CML444/MALAWI-342	6.27	11.45	CML444/MALAWI-318	2.66	7.49
	CML444/MALAWI-377	6.22	9.18	CML444/MALAWI-69	2.61	8.47
	CML444/MALAWI-327	6.20	9.56	CML444/MALAWI-187	2.52	7.67
	CML444/MALAWI-50	6.08	10.73	CML444/MALAWI-46	2.45	7.84
	CML444/MALAWI-117	6.07	9.17	CML444/MALAWI-186	2.25	5.97
	CML444/MALAWI-356	6.03	10.23	CML444/MALAWI-388	2.20	6.85
	Mean of lines	6.30	9.93		2.63	8.34
	Mean of population	4.64	8.58		4.64	8.58
CML440xCML504	CML440/CML504-B-439	6.35	9.95	CML440/COMPE2-B-186	4.03	7.29
	CML440/CML504-B-528	6.15	12.27	CML440/COMPE2-B-337	4.02	9.75
	CML440/CML504-B-551	6.10	10.12	CML440/COMPE2-B-429	3.95	7.79
	CML440/CML504-B-485	6.07	11.85	CML440/COMPE2-B-295	3.85	7.52
	CML440/CML504-B-559	6.00	11.05	CML440/COMPE2-B-21	3.85	10.59
	CML440/CML504-B-814	5.98	10.37	CML440/COMPE2-B-458	3.78	7.99
	CML440/CML504-B-292	5.91	9.82	CML440/COMPE2-B-414	3.70	10.43
	CML440/CML504-B-389	5.88	13.61	CML440/COMPE2-B-139	3.62	6.88
	CML440/CML504-B-597	5.83	11.60	CML440/COMPE2-B-469	3.55	8.59
	CML440/CML504-B-627	5.78	14.22	CML440/COMPE2-B-318	3.43	9.10
	CML440/CML504-B-633	5.75	12.24	CML440/COMPE2-B-375	3.38	10.66
	Mean of lines	5.98	11.55		3.74	8.78
	Mean of population	4.88	10.00		4.88	10.00
CML444xCML441	CML441/CML444-B-3	5.79	10.78	CML441/CML444-B-337	2.60	7.32
	CML441/CML444-B-454	5.25	11.92	CML441/CML444-B-735	2.58	9.12
	CML441/CML444-B-382	5.17	9.82	CML441/CML444-B-106	2.56	5.97
	CML441/CML444-B-548	5.04	10.78	CML441/CML444-B-615	2.52	10.16
	CML441/CML444-B-103	5.01	10.76	CML441/CML444-B-744	2.49	7.84
	CML441/CML444-B-68	4.92	11.01	CML441/CML444-B-813	2.45	5.77
	CML441/CML444-B-457	4.89	11.33	CML441/CML444-B-698	2.38	8.19
	CML441/CML444-B-7	4.88	12.12	CML441/CML444-B-550	2.29	9.24
	CML441/CML444-B-417	4.82	11.11	CML441/CML444-B-246	1.80	8.47
	CML441/CML444-B-46	4.80	9.67	CML441/CML444-B-690	1.38	3.27
	CML441/CML444-B-514	4.74	11.55	CML441/CML444-B-728	0.00	7.14
	Mean of lines	5.03	10.99		2.10	7.50
	Mean of population	3.66	9.91		3.66	9.91

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CHAPTER II

Unraveling QTLs affecting morphophysiological traits related to drought tolerance in three maize tropical populations

Abstract: Morphophysiological traits play an important role in drought adaptation in maize. Identifying Quantitative Trait Loci (QTLs) of sizeable effects that are expressed on diverse genetic backgrounds across contrasting water regimes can significantly complement conventional drought tolerance breeding efforts. We evaluated three tropical maize biparental populations under water-stressed (WS) and well-watered (WW) regimes to determine the behaviour and relationship traits, such as anthesis-silking interval (ASI), ears per plant (EPP), stay-green (SG), plant ear height (PEH) traits and grain yield (GY), and detect consistent genomic regions across different genetic backgrounds that could be target regions for drought tolerance in maize. On average, drought stress reduced the GY around to 50% and increased the ASI above 80% across three populations. Interestingly, stress had no affect on the other morphophysiological traits evaluated in this study. In general, drought stress tends to reduce the heritability of GY, while morphophysiological traits remain stable or increase under drought conditions. A single QTL analysis revealed a total of 203 QTLs for ASI, EPP, SG and PEH traits between both water regimes. Meta-QTL analysis across the three populations identified six constitutive genomic regions with a minimum of two overlapping traits. Clusters of QTLs were observed on chromosomes 1 (bin 1.06), 3 (bin 3.06), 4 (bin 4.09), 5 (5.05), 7 (bin 7.03) and 10 (bin 04-06). Interestingly, bin 3.06 harboured QTLs for all of the morphophysiological

traits included in this study. The genomic regions identified in this study might partially explain the association of secondary traits and grain yield, particularly under water limitation. However, it is uncertain whether many QTLs occur as a cluster in this region or if pleiotropy of a single genomic region is responsible for the manifold effects. Indeed, this mQTL region merits attention for further use in marker-assisted selection.

Keywords: secondary traits, metaQTLs, drought tolerance, SNP.

Introduction

Maize (*Zea mays* L.) is an important economic crop and is currently recognised as a major and eminent food security crop worldwide due its high yield potential (Ge et al. 2012). Water scarcity is the most important environmental limiting factor for maize productivity in tropical and subtropical regions (Messmer et al. 2011). It has been projected that by the year 2050, a 70% increase in global food production must occur. While the global climate change scenario tends to increase the problems of food insecurity (Varshney et al. 2011), this grim forecast has forced plant scientists to breed cultivars that can be grown in marginal areas. The benefits of genetic improvement for water stress are helpful, especially to poor farmers because tolerant genes for abiotic stresses are incorporated into the seed, and farms do not depend only of agronomic techniques to have yield productions (Duvick 2005). Drought stress can adversely affect many aspects of maize physiological metabolism and growth, including photosynthesis, plant height, dry matter production, leaf area and grain yield (Ge et al. 2012). Plants undergo various morphological, biochemical and physiological changes to respond and adapt in order to survive under drought stress (Lu et al. 2011).

Increasing grain yield (GY) is the primary objective of breeding for drought tolerance; however, the reduction of the genotypic variance of GY under drought stress through direct selection has hindered progress in yield and stability under water scarcity (Tuberosa et al. 2002). Some morphophysiological, or secondary, traits can experience increased genetic variance and heritability under stress conditions. It has been demonstrated that some secondary traits, such as anthesis-silking interval (ASI), ears per plant (EPP), plant height and stay-green traits (leaf senescence and chlorophyll content), remain stable under drought stress or might even increase (Bolaños and Edmeades 1996; Beltrán et al. 2003; Messmer et al. 2009; Lu et al. 2011; Messmer et al. 2011). Thus, these traits might be useful to improve selection efficiency for drought tolerance, and their use has been suggested for the improved tolerance of maize to drought and low-nitrogen conditions (Bänzinger and Lafitte 1997; Bänzinger et al. 2000; Beltrán et al. 2003; Lu et al. 2011). A desirable secondary trait should be genetically correlated with final grain yield, genetic variability, increased heritability, genetic diversity within the species, cost effective measurements, reliable assessments with individual plants or in small plots and no association with poor yield in unstressed environments (Monneveux et al. 2008; Ribaut et al. 2009; Lu et al. 2011).

The value of secondary traits for drought tolerance has been demonstrated through examining genetic correlations with GY or estimating the correlated response after indirect selection for GY (Bänzinger et al. 2000; Beltrán et al. 2003, Lu et al. 2011). In Mexico, Bolaños and Edmeades (1996) evaluated 3509 inbred lines among 50 traits under WW and WS conditions and detected a strong genotypic correlation at a magnitude of -0.60 and 0.90 for ASI and EPP, respectively. Chapman and Edmeades (1999) detected a genotypic correlation of -0.89, 0.95 and 0.70 between

GY and ASI, EPP and the visual leaf senescence score, respectively, under drought condition. More recently, Lu et al. (2011) reported the genetic correlation of many secondary traits under WW and WS, evaluating one set of 550 temperate, tropical and subtropical recombinant inbred lines (RILs) from CIMMYT (International Maize and Wheat Improvement Center) and CAAS (Chinese Agricultural Academic Science). These authors detected a positive and significant association between GY and plant height, chlorophyll content in ear leaves and leaf senescence under drought conditions and optimal water supply. Moreover, the genetic gain for grain yield could be higher with the use of secondary traits in a combined index selection than in the case of selection in grain yield *per se*. The selection efficiency in 19 maize populations under low nitrogen conditions was improved by 14% when secondary traits, such as ASI, EPP and stay-green, were included in the index selection over selection for grain yield alone (Bänzinger and Lafitte 1999).

Maize is more susceptible than other rain-fed crops because of its near-synchronous development of florets, usually on a single ear, and the physical separation of male and female flowers on the same plant, which exposes silks and pollen. Typically, the anthesis date (AD) is slightly affected under drought conditions, while this stress promoting slow silk growth and consequently a long ASI, which is an external indicator of reduced partitioning to the ear (Araus et al. 2011). Leaf senescence is a type of cell death programme that is inappropriately activated in response to the degradation of chlorophyll in plants under drought conditions. Delayed leaf senescence and higher chlorophyll concentrations are associated with the stay-green capacity of plants and play an important role in enhancing drought tolerance (Rivero et al. 2008). Stay-green genotypes are associated with the retention of chlorophyll in the leaves and maintenance of the ability to undergo photosynthesis

for longer periods than senescent genotypes under terminal drought conditions (Harris et al. 2007). Stay-green could be evaluated at the leaf level using portable chlorophyll metres, such as the Minolta SPAD (Cai et al. 2012), in accordance with Ribaut et al. (2009), who previously proposed that secondary traits should be low cost and easily measured. However, published QTLs studies concerning the genetic control of this trait in maize influenced by drought stress are scarce (Messmer et al. 2011). Root traits play an important role in plant adaptation to drought-prone conditions. However, selecting root traits in maize is difficult (Hund et al. 2011), especially for genomic approaches where an elevated number of genotypes should be screened (Landi et al. 2010). Then, non-destructive measurement of root capacitance using a portable capacitance metre offers a feasible way of approximating the relative differences in the extension of the root system (Rajakai et al. 2005). However, the efficiency of root capacitance for maize drought tolerance breeding has been questionable (Lu et al. 2011; Messmer et al. 2011).

An association between morphophysiological traits and yield components and their use in conventional breeding programmes has been frequently demonstrated (Bolaños and Edmeades 1996; Bänzinger and Lafitte 1999; Beltrán et al. 2003; Monneveux et al. 2008; Zheng et al. 2009; Lu e t al. 2011). However, these studies have provided insufficient information concerning how chromosome position regulates the variation of each trait, the genetic basis for the simultaneous effects of each chromosome region on other traits and the interpretation of possible case-effect relationships among traits (Tuberosa et al. 2002). Molecular marker approaches offer an important tool to understand the relationship between grain yield and secondary traits (Messmer et al. 2011). Indeed, the co-localisation of QTLs with secondary traits has not been well defined using phenotypic analyses. This can assist breeders in the

identification of hotspot genomic regions for use in marker-assisted selection (MAS) programmes (Landi et al. 2010). Moreover, an important prerequisite for a successful MAS programme aimed at improving drought tolerance is the identification of QTLs that consistently affect crop growth and performance, particularly yield, across different water regimes (Collins et al. 2008) and genetic backgrounds (Swamy et al. 2011).

We present the first study concerning QTL mapping in maize, using three tropical biparental populations and Single Nucleotide Polymorphism (SNP) markers for the detection of precise genomic regions at physical positions on the genome for secondary traits in order to understand their genetic association of these traits and grain yield at the genome level. This study addressed the following aims: (1) to determine the behaviour and relationship of secondary traits with GY under stressed and non-stressed water environments; (2) to identify the genomic regions responsible for the expression of secondary traits across water regimes; and (3) to detect consistent genomic regions across different genetic backgrounds that could be hotspots for drought tolerance in maize.

Materials and methods

Plant materials

Three biparental maize populations from Global Maize Program-CIMMYT were evaluated under well-watered (WW) and water-stressed (WS) conditions. *Population 1(tolerant x intermediary susceptible)*: Comprised 234 recombinant inbred lines (RILs) from the cross CML444xMALAWI developed using the single seed descent method. *Population 2 (tolerant x tolerant)*: Comprised 247 $F_{2:3}$ families from the cross CML440xCML504, obtained from randomly chosen F_2 plants. *Population 3*

(tolerant x tolerant): Comprised 300 $F_{2:3}$ families, obtained from randomly chosen F_2 plants from the cross CML444xCML441. The parental lines CML444, CML441, CML440 and CML504 adapted to tropical and subtropical African mid-altitude environments and were considered to be tolerant to drought and low-nitrogen levels. These lines have a compact phenotype with strong, erectophile and dark green leaves. SC-MALAWI is also a subtropical line with moderate tolerance to water-limited conditions, but these plants exhibit long horizontal and light green leaves. This inbred line was developed in southern Zimbabwe in the 1960s and has been widely used in crosses for developing public and private hybrids for mid-altitude. Segregating families of CML444xMALAWI and CML444xCML441 were test crossed to CML312, whereas CML440xCML504 was test crossed to CML395 for phenotypic evaluations.

Field experiments

The field experiments were conducted in Mexico (Tlatizapán station: 18ºN, 99ºW, 940 m). One field experiment was conducted under well-watered (WW) conditions during the rainy season in 2010, and two field experiments were conducted under water-stressed (WS) conditions during the dry season in 2010 and 2011 for each population. Climatologic conditions of this environment for drought phenotyping have been previously described (Masuka et al. 2012). The experimental design was an alpha (0,1) lattice (Patterson and Williams 1976) with two replications and one-row plot size of 5 m, with 0.75 m between the rows. Plots were planted with two seeds per hill and thinned to one plant per hill three weeks after planting, resulting in a plant population of approximately 66,667 plants ha^{-1}. Drought stress was applied during the flowering time in accordance with the established protocols in CIMMYT (Bänziger et

al. 2000). For water-stressed conditions, the furrow irrigation method at 10-day intervals was used until three weeks before the expected anthesis date (AD) in each population. This stress condition was maintained until five weeks after 50% of the families flowered. An additional irrigation was applied during grain filling. In WW trials at all the locations, the soil moisture was maintained at field capacity.

The traits evaluated in this study were consistent with CIMMYT's uniform standardised protocols (Bänziger et al. 2000; Betrán et al. 2003; Araus et al. 2011; Lu et al. 2011). A total of nine traits were measured under both water regimes. The trait names and brief measurement descriptions are listed in Table 1. Traits with more detailed measurements are described below. Senescence and relative chlorophyll content were measured three times (every two weeks) after three weeks 50% of the families reached AD. The three measurements were used to estimate the areas under curve of progress of senescence and chlorophyll content. Root capacitance were measured once in five plants per plot using a BK Precision 810A Meter (Maxtec Inc., Chicago, USA); the negative electrode was connected to the stem above the first node, and the positive electrode was connected to a rod inserted into the soil in the middle section of the furrow next to the plot under consideration (Messmer et al. 2011; Lu et al. 2011).

Table 1. Description of the measured traits for drought tolerance

Traits[1]	**Description**
GY	Grain yield in t/ha
ASI	Anthesis-silking interval, measured as the difference between male (AD) and female flowering (SD) time, the interval time from sowing to 50% Individuals flowering in each plot.
EPP	Number of ears per plant, measured as number of harvested ears with kernels by the number of plants per plot
SENES	Leaf senescence, scored using a scale from 0 to 10 (1 = 10%; 2 = 20%; 3 = 30%; 4 = 40%; 5 = 50; 6 = 60%; 7 = 70%; 8 = 80%; 9 = 90% and 10 = 100% dead leaf area scored at three, five and seven weeks after 50% of the plant reached anthesis.
CEL	Chlorophyll content in the ear leaves, measured in five plants per plot at three, five and seven weeks after 50% of the plants reached at anthesis using a SPAD metre.
CYL	Chlorophyll content in young leaf (second leaf from tassel) measured in five plants per plot at three, five and seven weeks after 50% of the plants reached anthesis using a SPAD metre.
PH	The average height from ground to the tassel tip in five plants scored randomly in each plot.
EH	The average height from ground to the node bearing the highest ear in five plants scored randomly in each plot.
RC	Root capacitance, measured using an electrical capacitance metre at two days after a grain filling irrigation

[1] Abbreviation of traits names.

Phenotypic data analysis

The raw plot data were analysed in linear mixed model in PROC Mixed of SAS using REML as described in chapter 1. In WS conditions, the two field experiments per population were analysed using a combined analysis. The adjusted means for each line were estimated using the following linear model: $Y_{ijk} = \mu + \mathrm{Re} + B_j(\mathrm{Re}) + G_k + \varepsilon_{ijk}$, where, Y is the trait of interest, μ is the mean effect, Re is the effect of the ith replicate, $B_j(\mathrm{Re})$ is the effect of the jth incomplete block within the ith replicate and G_k is the effect of the kth genotype. In the case of combined data from two year terms, E_i and $(E_i \times G_l)$ were incorporated into the linear model, where, E_i is the effects of the ith environment and $(E_i \times G_l)$ represents the environment x genotype interaction. To estimate the Best Linear Estimated Value (BLUEs), the genotypes were considered as fixed terms, while all other terms were declared random. The broad-sense heritability (H^2) was estimated using the formula: $H^2 = \sigma^2_{\mathrm{G}}/(\sigma^2_{\mathrm{G}} + \sigma^2_{\mathrm{GE}}/\mathrm{l} + \sigma^2/\mathrm{lr})$, where σ^2_{G} is the genotypic variance, σ^2_{GE} is the genotype x environment interaction, σ^2 is the error variance, (l) is the number of environments and (r) is the number of replications in each trials. The genetic correlations among traits corresponded to the ratio between the genotypic covariance for each pair of traits and the product of the respective genotypic standard deviation. The phenotypic correlations among traits were calculated as simple Pearson's correlation coefficients based on adjusted phenotypic data.

QTL identification

Individual linkage maps for each population were constructed using QTL IciMapping ver. 3.2, as describe in Chapter 1. A brief description of the linkage maps in each

population is given below. For the RILs population CML444xMALAWI, one linkage map with 2,349.23 cM was constructed using the allelic information from 216 SNPs and 160 SSR markers that were previously reported (Messmer et al. 2009, 2011; Trachsel et al. 2010). In the $F_{2:3}$ populations CML440xCML504 and CML444xCML441, linkage maps were constructed using 194 and 265 SNPs markers covering a total of 2,712.30 and 3,558.33 cM of the maize genome, respectively. To identify common genomic regions across the populations, three distinct genetic maps were merged into a single integrated map using MetaQTL software version 1.0 (Veyrieras et al. 2007). The distances between the adjacent markers from all individual maps were rescaled in Haldane units. After integrating the three maps, a consensus map of 620 markers was obtained. The consensus map had a total length of 1,484.45 cM with an average distance of 2.39 cM between the markers (Fig. 1).

Single QTL mapping analyses were performed for EPP, SENES, CEL, CYL, EH and PH in both water regimes. QTL mapping for GY and ASI in these three biparental populations was previously performed in Chapter 1. Root capacitance was not included in the QTL mapping study due the lack of association between this trait and GY under WS conditions. The QTLs were identified for the adjusted means for each trait using Inclusive Composite Interval Mapping (ICIM) (Li et al. 2007) implemented in the integrated software QTL IciMapping v.3.2 (http://www.isbreeding.net). In all populations, the walking step in QTL scanning was 1 cM, and a likelihood odds (LOD) threshold of 2.5 was chosen for declaring potentially significant QTLs for drought tolerance (Ribaut et al. 1997; Tuberosa et al. 2002). For $F_{2:3}$ populations, the additive (A) and dominance (D) effects for each QTL were given by the programme and used to calculate the ratio of the dominance level |D/A| and classified accord criteria according to Stuber et al. (1987). The QTLs were

considered as additive (A) = 0–0.20; partial dominance (PD) = 0.21–0.80; dominance (D) = 0.81–1.20; and overdominance OD > 1.20. The sign of the additive effects of each QTL was used to identify the origin of the favourable alleles in accordance with Lubberstedt et al. (1997). The lines used in all three crosses with positive signs were CML444 and CML440, and MALAWI, CML504 and CML441 were considered for the crosses with negative signs. These signals reflected the agronomic performance of the line under drought conditions. However, in populations CML440xCML504 and CML444xCML44, both parents exhibited high agronomical performances in drought conditions.

Meta QTL analysis was performed using the several identified QTLs using a single QTL analysis in each environment with the MetaQTL software version 1.0 (Veyrieras et al. 2007). Using this analysis, it is possible to identify genomic regions that can be considered as hotspots for marker-assisted selection because single QTLs for each trait detected in different mapping population experiments can be estimated at the same chromosomal region (Swamy et. 2011). The well-correlated traits were merged into a single trait in meta-analysis in the trait ontology tree. Senescence and chlorophyll content in the ear and young leaves were described as a single trait called stay-green (SG), PH and EH were declared as the plant/ears height ratio (PEH) and EPP was described as a single trait. Meta-QTLs for GY and ASI were obtained from previous results (Chapter 1). Only regions harbouring a single QTL from three populations or a higher number of QTLs from a minimum of two populations were considered as meta-QTLs. Detailed explanations concerning the meta-QTL analysis were previously described (Danan et al. 2011).

Results

Trait variation and correlations under two water regimes

The estimated means and heritability for each trait in all three populations are listed in Table 2. We observed from WW to drought conditions a marked-reduction in yield in the RILs and $F_{2:3}$ families. The yield reductions in the populations CML444xMALAWI, CML440xCML504 and CML444xCML441 were approximately 46%, 40% and 49%, respectively. In all three populations, the genetic variance of GY in WS was smaller than that in WW. The heritability under WS (h^2_{GYws}) ranged from 0.22 to 0.54, while the heritability under WW (h^2_{GYww}) ranged from 0.46 to 0.72. The heritability values for EPP were stable under both water regimes. The heritability under WW (h^2_{EPPww}) ranged from 0.00 to 0.34, and the heritability under stress (h^2_{EPPws}) ranged from 0.09 to 0.31. The null h^2_{EPPww} in population CML444xMALAWI is due to the reduced variation in this trait; almost all genotypes from this population contained one ear per plant (Table 2). A major reduction in EPP from to WW to WS was detected in population CML444xMALAWI (13%), whereas these reduction values were 7% and 5% for CML440xCML504 and CML444xCML441, respectively (Table 2). This result potentially reflects the high performances of these populations under water limitation.

The interval between male and female flowering (ASI) was highly increase under water shortage. Across three populations, the average of ASI under WW was 0.64, while under WS, the average ASI was 3.52, indicating an increase in the anthesis-silking interval of greater than 80%. However, under water limitation conditions, the genetic variance of this trait increases substantially. The heritability values under WW (h^2_{ASIww}) ranged from 0.25 to 0.47, while under WS (h^2_{ASIws}), the values ranged from 0.30 to 0.63 (Table 2). Similar behaviours for the stay-green traits

(SENS, CEL and CYL) were observed. The heritability values for leaf senescence and chlorophyll content in the ear leaves in all three populations were higher under WS conditions than under WW. In general, the magnitude of heritability for ASI, SENES and CEL traits tends to was higher than that for GY under water limitation. This response has important implications for incorporating secondary traits into the selection criteria to increase selection efficiency under drought conditions.

Table 2. Means and heritabilities (h^2) for trait tested under well-watered (WW) and water-stressed (WS) conditions among three biparental populations

		CML444xMALAWI		**CML440xCML504**		**CML444xCML441**	
Trait	Treat.	Mean	h^2	Mean	h^2	Mean	h^2
GY	WW	9.57	**0.46**	8.61	**0.73**	10.81	**0.72**
(t/ha)	WS	5.11	**0.22**	5.19	**0.28**	5.52	**0.54**
ASI	WW	0.74	**0.39**	0.84	**0.25**	0.35	**0.47**
(days)	WS	3.16	**0.30**	3.53	**0.38**	3.89	**0.63**
PH	WW	246.94	**0.63**	232.36	**0.62**	253.38	**0.50**
(cm)	WS	223.70	**0.40**	211.86	**0.52**	215.76	**0.53**
EH	WW	139.95	**0.79**	138.28	**0.45**	139.38	**0.73**
(cm)	WS	128.35	**0.52**	118.24	**0.56**	115.60	**0.72**
EPP	WW	0.99	**0.00**	0.97	**0.26**	0.98	**0.34**
(uni)	WS	0.86	**0.09**	0.90	**0.22**	0.93	**0.31**
SENES	WW	3.63	**0.07**	4.69	**0.20**	6.64	**0.28**
(uni)	WS	7.63	**0.27**	8.21	**0.27**	7.65	**0.44**
CEL	WW	12.77	**0.05**	12.40	**0.26**	14.59	**0.27**
(uni)	WS	11.64	**0.37**	11.09	**0.30**	12.75	**0.40**
CYL	WW	9.78	**0.07**	8.52	**0.09**	10.87	**0.18**
(uni)	WS	7.87	**0.16**	6.47	**0.00**	87.51	**0.51**
RC	WW	3.99	**0.33**	2.25	**0.00**	4.36	**0.47**
(200nF)	WS	6.67	**0.35**	7.24	**0.22**	6.73	**0.26**

SENES, CEL and CYL are expressed as the area under the curve based on three measurements divided by one constant.

The phenotypic (r_p) and genotypic (r_g) correlations between GY and morphophysiological traits are listed in Table 3 and 4, respectively. In some traits, it was not possible to estimate genotypic correlation values because of negative or null values of genetic variance of one trait, which was identified as NA^{ϕ} (Table 4). The anthesis-silking interval is negatively correlated with GY. Under WS, the correlation is high and significant, whereas, these two traits were weakly or not correlated under WW. This result was consistent with previous reports (Bolaños and Edmeades 1996; Beltrán et al. 2003) indicating that ASI is a secondary trait that provides the most important adaptive mechanism for drought tolerance in maize. A delayed silk emergence occurs in response to reduced kernel size. The increase in the ASI occurs with a concomitant reduction in the number of EPP. These two traits showed medium to high genetic correlations of -0.46, -0.89 and -0.23 for CML444xMALAWI, CML440xCML504 and CML444xCML441, respectively (data not shown). During water limitation, EPP is directly related to reduce barrenness, which can be confirmed by the increased correlation between EPP and GY. Senescence was negatively correlated with GY across three populations under both water regimes. Whereas, a positive correlation between chlorophyll content (CEL and CYL) and GY was observed (Table 3, 4), suggesting that stay-green traits are essential to obtain genotypes that perform well under both water regimes. The radiation use efficiency is higher in genotypes with high chlorophyll content, which reflects the ability of the plant to capture more solar energy for a longer period during grain filling when senescence is delayed. Drought stress during grain filling accelerates leaf senescence, and genotypes that maintain functional leaf areas are more capable of filling kernels. The increased chlorophyll content was reflected in an increase in EPP under drought stress. The genotypic correlations between EPP and CEL under WS conditions were

0.70, 0.51 and 0.91 for CML444xMALAWI, CML440xCML504 and CML444xCML441, respectively (data not shown). Overall, in all three populations, the correlations between chlorophyll and GY were stronger than those with senescence (visual scores), which indicates the importance of high precision phenotyping for drought tolerance breeding (Masuka et al. 2012).

Water stress slightly tends to reduce the average and heritability of PH and EH (Table 2). In addition, the PH and EH were better correlated with GY under WS than under WW (Table 3 and 4). Under drought, tall plants apparently had a greater capacity for grain filling than shorter plants, most likely because of larger photosynthetically active leaf areas and greater stem reserves.

A significant correlation between GY and root capacitance was not observed under WS, and a weak association between these two traits was observed under WW (Table 3, 4). Moreover, this trait is not inheritable, contrary to the other morphophysiological traits evaluated in this study (Table 2). Thus, for QTL mapping approaches, we decided to only use the secondary traits that play important roles in drought adaptation in maize, not including root capacitance.

Table 3. Phenotypic correlations between the grain yield (GY) and test traits under well-watered (WW) and water-stressed (WS) conditions among four biparental populations

Population	Treat.	Traits							
		ASI	EPP	SENES	CEL	CYL	PH	EH	RC
CML444xMALAWI	WW	-0.06^{ns}	0.13^{ns}	-0.11^{ns}	0.40***	0.34***	0.37***	0.17^{ns}	-0.27***
	WS	-0.51***	0.44***	-0.07^{ns}	0.28***	0.20*	0.36***	0.26***	0.11^{ns}
CML440xCML504	WW	-0.18*	0.38***	-0.36***	0.37***	0.28**	0.40***	0.34***	0.17*
	WS	-0.28***	0.59***	-0.17**	0.30***	0.31***	0.33***	0.20**	0.01^{ns}
CML444xCML441	WW	-0.23**	0.44***	-0.32***	0.30***	0.29***	0.13*	0.12^{ns}	-0.17*
	WS	-0.28***	0.46***	-0.46***	0.60***	0.50***	0.40***	0.44***	0.08^{ns}

*, ** and *** indicate significance levels at 5%, 1%, 0.1%, respectively, and ns indicates non-significance. [1]Abbreviations are given in Table 1.

Table 4. Genotypic correlations between the grain yield (GY) and test traits under well-watered (WW) and water-stressed (WS) conditions among four biparental populations

Population	Treat.	Traits[1]							
		ASI	EPP	SENES	CEL	CYL	PH	EH	RC
CML444xMALAWI	WW	-0.14^{ns}	NA$^{\phi}$	-0.12^{ns}	0.69***	0.53**	0.70***	0.28^{ns}	-0.69***
	WS	-0.89***	NA$^{\phi}$	-0.12^{ns}	0.76***	0.59**	0.60***	0.40***	0.25^{ns}
CML440xCML504	WW	-0.42*	NA$^{\phi}$	-0.89***	0.86***	NA$^{\phi}$	0.61***	0.58***	NA$^{\phi}$
	WS	-0.86***	NA$^{\phi}$	-0.63***	NA$^{\phi}$	NA$^{\phi}$	0.86***	0.49**	0.05^{ns}
CML444xCML441	WW	-0.38*	0.87***	-0.71***	0.66***	0.79***	0.22^{ns}	0.16^{ns}	-0.28^{ns}
	WS	-0.45***	0.77***	-0.83***	NA$^{\phi}$	0.79***	0.61***	0.63***	0.24^{ns}

*, ** and *** indicate significance level at 5%, 1%, 0.1%, respectively and ns indicates non-significance. [1]Abbreviations are given in table 1.

QTL mapping for secondary traits under two water regimes

A total of 203 QTLs within the maize genome were revealed using a single-QTL analysis for ASI, EPP, SENES, CEL, CYL, EH and PH, under both water regimes, among the three populations with varying magnitudes (Table S1, S2 and S3).

However, approximately 60% of the QTLs were detected under WS, which confirms the importance of secondary traits in breeding programme for drought tolerance. Both parental lines in each population contributed positive alleles for all traits evaluated, potentially reflecting the use of lines with good performance under drought conditions. Chromosomes 1, 3 and 10 harboured the largest number of QTLs in both water regimes. However, only chr.3 harboured QTLs for all morphophysiological traits under WS and WW conditions (Table S1, S2, S3, Fig. 1 and Fig. S1). The QTLs for GY and ASI under WW and WS in Mexico were previously identified in Chapter 1. In the section below we will present only the QTL results for ears per plant (EPP), stay-green traits (SENES, CEL and CYL) and plant and ear heights (PH and EH). However, to identify QTL clusters in some maize genome regions, we included GY and ASI from previous studies (Chapter 1).

QTLs for EPP

A total of 29 QTLs were detected for EPP among all three populations under both water regimes. Even the additive effects of the QTLs for this trait were small due the small phenotypic variance of this trait (ranged from 0.0005 to 0.18 ears), and major QTLs (R^2> 10%) were detected in the $F_{2:3}$ populations. In the CML440xCML504 population, two genomic regions were detected under WW conditions on chr.1 (64.26-76.05 Mb and 90.77-90.78 Mb), and one region was detected under WS conditions on chr.5 (206.33-208.90 Mb) (Table S2). In the CML444xCML441 population, one QTL on chr.10 was detected (99.47-120.54 Mb), which explained 32.28% of the phenotypic variance under WS conditions (Table S3).

QTL for stay-green traits

The high correlation between the SENES, CEL and CYL might explained by a large part of the QTLs for these three traits were co-localized, particularly under WS. However, an evaluation of the visual senescence and leaf chlorophyll contents allowed the identification of more regions that were responsible for stay-green traits in maize. A total of 80 QTLs were detected for SENES, CEL and CYL under both water regimes among three biparental populations (Table S1, S2 and S3). There was a slightly higher number of QTLs identified under WS conditions (51%) than under WW conditions (49%), especially for SENES. This result potentially reflects a higher correlation between SENES and GY under WS than under WW (Table 3, 4). When we consider only the RILs population CML444xMALAWI, no QTLs were detected under WW conditions. For leaf senescence, major QTLs were detected in all three populations only under WS conditions, indicating the importance of this trait as a drought adaptation mechanism. The CML444xMALAWI population contained one QTL on chr.4 (104.16-128.63 Mb) explaining 15.75% of the phenotype variance (Table S1). For the $F_{2:3}$ populations, one QTL on chr.10 (120.54-146.55 Mb) was revealed in CML440xCML504, and two QTLs were detected on chr.2 (195.55-195.93 Mb and 197.10-199.41 Mb) in population CML444xCML441, explaining 13.25% and 21.05% of the phenotypic variance, respectively (Table S2, S3). With regard to the chlorophyll content, one QTL on chr.3 (52.80-210.16 Mb) was detected in the CML444xCML441 population under WS conditions, explaining 15.71% and 20.71% of the phenotypic variance for CEL and CYL, respectively (Table S3).

QTL for PH and EH

A total of 62 QTLs were detected for PH and EH in all three populations under WW and WS regimes. The QTLs were located across all chromosomes for both traits, except on chr.10, which did not harbour any QTLs for PH. However, in all three populations QTLs for EH were detected on bin. 10.04-06 under both water conditions (Table S1, S2, S3 and Table 5). Both parental lines in each population contributed with positive QTL alleles. Considering the two populations where CML444 is a common parental line (CML444xMALAWI and CML444xCML441) 55% and 70%, respectively, of the QTLs for these two traits were originally from CML444 (Table S1, S3). With regard to CML440xCML504, the line CML504 contributed 55% of the alleles for these traits (Table S2). Unlike in RILs, the heritability magnitude in the $F_{2:3}$ populations of these two traits under WS was higher or similar to the values obtained under WW (Table 2). Most of the major QTLs were detected under WW conditions. One QTL was detected in the CML440xCML504 population for PH under WW conditions on bin 1.08 (217.50 -239.31 Mb), which was derived from CML504, explaining 30.55% of the phenotype variance. Under water scarcity, one QTL on chr.5 (97.98-167.87 Mb) was detected, explaining 10.98% of the variance in the CML444xCML441 population for PH. Another region in chr.7 (129.79-134.85 Mb) was detected in the CML440xCML504 population, explaining 10.56% of the variance under WS conditions.

Clusters of QTLs detected by meta-analysis

Of the 203 QTLs detected for all secondary traits under both water regimes (Table S1, S2 and S3), we plotted 174 onto a consensus map of the three populations to perform a meta-QTL analysis (mQTL). The remaining QTL intervals that were not supported

by a minimum of two anchor markers and QTLs explaining less than 2% of the variance were excluded from the analysis. A meta-QTL was declared only when it was common to all three biparental populations or when one region harboured an elevated number of QTLs derived from a minimum of two populations. The meta-QTL analysis enabled the detection of target regions for drought tolerance because clusters of QTLs for traits that were not well correlated could be detected overlapping the same region. Therefore, hotspot regions harbouring a large number of traits can be useful in an MAS programme for drought tolerance. In this study, we identified eight mQTLs for stay-green traits (SG), two for ears per plant (EPP) and four for plant ears height (PEH), respectively, with a confidence interval of 95% (Table 5). We plotted in consensus map from the three populations (Fig. 1) a set of mQTLs for secondary traits identified in this study (SG, EPP and PEH); additionally, meta-QTLs for GY and ASI were noted, as previously described (Chapter 1). A meta-QTL for stay-green was detected on chromosomes 1, 3, 4, 5, 8 and 10. Two SG-mQTLs each were identified on chr.1 and chr.5 and one was detected on chr.3, chr.4, chr.8 and chr.10. One EPP-mQTL was detected on chr.2 and chr.3. PEH-mQTLs were identified on chr.1, chr.3, chr.7 and chr.10. The confidence intervals for the 14 mQTLs identified in this study ranged from 2.30 to 23.32 cM. These values are below the previously established arbitrary threshold of 30 cM (Hund et al. 2011). In addition, we also provided the physical intervals of mQTLs to compare them with previous results for use in marker-assisted breeding and the identification of candidate genes co-locates in these regions (Table 5). Considering only stay-green, the mQTL on chr.3 (mQTL_SG_3) had the largest number of QTLs integrated from all three populations under WW and WS conditions. For the plant ear height (PEH), the mQTL on chr.7 (mQTL_PEH_7) harboured seven QTLs under WW and WS conditions, and for EPP, both mQTLs

integrated the same number of QTLs. All 14 mQTLs for SG, EPP and PEH were integrated by the QTL derived from both water regimes, indicating that those regions might play an important role in conferring a constitutive drought response in maize (Table 5).

Most of the mQTLs for secondary traits overlapped with previously identified adaptive or constitutive regions responsible for the GY detected across all three populations (Chapter 1). Only EPP-mQTL did not overlap with any GY-mQTLs (Table 5 and Fig. 1). Constitutive genomic regions regulating grain yield on chr.4 (242.02-244.10 Mb) and chr.5 (171.69-199.70 Mb) overlapped stay-green mQTL_SG_4 and mQTL_SG_5b. While the adaptive region on chr.7, mQTL_GY_7 (123.61-132.28 Mb) overlapped with the meta-QTL for plant ear height (mQTL_PEH_7). Two genomic regions harboured mQTLs for grain yield concomitant with stay-green and plant ear height traits. The first mQTL, located on bin 1.05/06 (161.07-183.83 Mb), overlapped with the mQTL_SG_1b and mQTL_PEH_1. The second mQTL, located on chr.10 (121.49-147.76 Mb), overlapped with mQTL_SG_10 and mQTL_PEH_10 (Table 5 and Fig. 1). No GY-meta-QTLs were detected on chr.3. However, Chapter 1 previously described the genomic region on chr.3 (169.75-178.23 Mb) as an important adaptive region regulating ASI under drought conditions, indicating an important role for morphophysiological traits in maize. Similarly, this region overlapped with meta-QTLs for all secondary traits involved in this study (Table 5, Fig. 1 and Fig. S1), indicating the importance of this genomic region for adaptive and constitutive mechanisms of secondary traits for drought tolerance.

Table 5. Meta-QTLs for stay-green (SG), ears per plant (EPP) and plant ear height (PEH) traits across three populations identified using meta-analysis.

Trait	mQTL[1]	Bin	Pos. (cM)	Confidence interval (cM)	Flaking markers	Physical interval (Mb)	QTL number	QTL integrated[2]
SG	mQTL_SG_1a	1.03	129.14	120.05-138.23	pza02376.1-bnlg2238	44.36-55.08	4	*pop2CYL_WS1, pop3CEL_WW1b, pop3Sen_WW1b, pop3Sene_WS1b*
	mQTL_SG_1b	1.05/06	174.06	170.87-177.25	pza02741.1-phm5622.21	161.07-183.83	4	*pop2SenWW1a, pop3Sen_WW1a, pop3Sen_WS1b, pop3CYL_WS1a*
	mQTL_SG_3	3.06	101.3	98.49-104.0	pza02212.1-umc7	169.75-178.23	8	*pop3CYl_WS3, pop2SenWW3, pop3SenWW3,pop2CEl_WW3a, pop1CYl_WS3, popCEL_WS3, pop2CEL_WW3b, pop3CEl_WW3*
	mQTL_SG_4	4.09	98.6	92.52-104.66	pza00529.4-phm4310.112	240.77-244.08	4	*pop2CYL-WW4, pop1Sen_WS4b, pop3CYl_WS4, pop2CEl_WW4*
	mQTL_SG_5a	5.04	85.02	80.58-89.46	pzb01017.1-pza00148.3	158.03-164.23	6	*pop2CYl_WS5a, pop3Sen_WS5, pop3CEl_WS5, pop2Sen_WW5, pop2CEl_WW5, pop3CYl_WS5*
	mQTL_SG_5b	5.05	129.72	124.48-134.97	phm13696.11-pza01142.4	175.3-199.69	4	*pop2CYl_WS5b, pop3CYl-WW5, pop3CEl_WW5, pop2CYl_WW5*
	mQTL_SG_8	8.06	46.02	40.57-51.47	pmh15278.6-asg52a	155.48-159.76	4	*pop3Sen_WS8, pop1Sen_WS8, pop2Sen_WW8, pop2CEl_WW8*
	mQTL_SG_10	10.04/06	43.43	40.24-46.61	pza01919.2-pza03607.1	111.26-141.82	6	*pop3Sen_WS10, pop3CEl_WW10, pop3CYl_WW10a, pop3Sen_WW10, pop3CYl_WS10, pop1Sen_WS10,*
EPP	mQTL_EPP_2	2.08/09	116.46	115.20-117.72	pza02012.7-pza02727.1	218.28-227.92	3	*pop1EppWS1, pop2Epp_WW3, pop3Epp_WS2a*
	mQTL_EPP_3	3.06/07	100.2	96.05-104.3	pzd00027.2-umc63a	169.75-214.41	3	*pop1EppWW3, pop2Epp_WS3a, pop3EppWS3*
PEH	mQTL_PEH_1	1.05	164	161.22-166.77	csu1138.4-pza02741.1	119.01-161.08	6	*pop1PH_WS1, pop2PH_WW1a, pop2EH_WS1, pop2EH_WW, pop3EH_WS1, pop3EH_WW*
	mQTL_PEH_3	3.06	97.49	94.84-100.14	pza00186.4-phm17210.5	165.80-178.22	5	*pop1EH_WW_3, pop1WS3b, pop2PH_WS3, pop3PH_WS3, pop3EHWS3*
	mQTL_PEH_7	7.03	25.01	23.93-26.31	pzb00752.1-pza02854.13	131.10-137.83	7	*pop1EH_WW7, pop3EH_WS7, pop2PH_WS7, pop2EH_WS7, pop3PH_WS7, pop2EH_WW7, pop1PH_WW7*
	mQTL_PEH_10	10.05/06	69.8	58.14-81.46	npi232a-pza02527.2	130.59-148.48	4	*pop1EH_WS10, pop1EH_WW10, pop2EH_WS10, pop3EH_WW10b*

[1] Meta-QTLs for stay-green (SG), ears per plant (EPP) and plant and ear height (PEH) followed by the chromosome number. [2] Detected QTLs using a single QTL analysis in each population across well-watered (WW) and water-stressed (WS) conditions. The three populations were represented in the following order: pop1 (CML444xMALAWI), pop2 (CML440xCML504) and pop3 (CML444xCML441). Mb= megabase (10^6 pb).

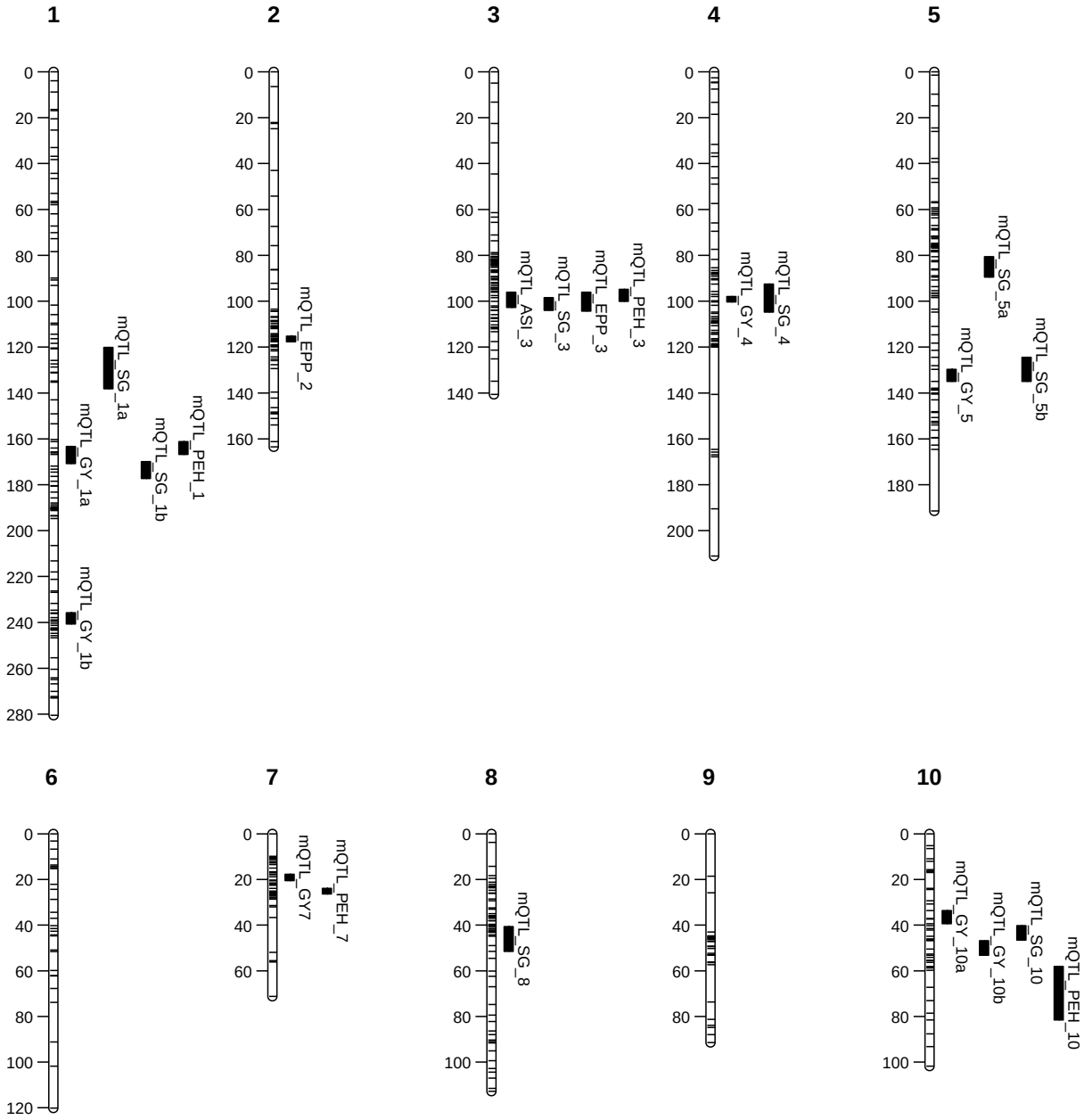

Fig. 1. Meta-QTLs for grain yield (GY), anthesis-silking interval (ASI), ears per plant (EPP), stay-green (SG) and plant ears height (PEH) traits in consensus map of the three maize tropical populations: CML444xMALAWI, CML440xCML504 and CML444xCML441. The meta-QTLs for GY and ASI were previously detected (Chapter 1).

Discussion

The expression of tolerance depends greatly on both, the developmental stage during which water stress is imposed and the intensity and duration of drought. Drought stresses imposed in all three biparental maize populations effectively decreased grain yield by approximately 50%. Such levels of stress falls occur within the range of stress levels reported in other studies aimed at improving drought tolerance in maize (Bolaños and Edmeades 1996; Chapman and Edmeades 1997; Messmer et al. 2009; Lu et al. 2011). According to Bänzinger et al. (2000), the yield reduction due to drought stress should be approximately 50%. Yield reductions of up to 70% drastically reduce the genetic variance component to almost null values, which subsequently reduces the detection of tolerant genotypes to water limitation (Monneveux et al. 2006; Araus et al. 2011). It demonstrates that the screening and selection of genotypes for drought tolerance in experiments using irrigation and plant date to control the timing and severity of stress during flowering in rain-free environments is an effective alternative to obtain genetic gain under drought conditions (Ribaut et al. 2009). In addition, these methods could increase the detection of genomic regions that are responsible for drought tolerance in maize.

The pronounced reduction in GY heritability under WS was observed in all three maize populations. The reduction in GY heritability has been well documented in breeding processes aimed at abiotic stress tolerance as reviewed by Ribaut et al. (2009). Moreover, the genetic variance of morphophysiological traits remains stable or increases under drought conditions (Beltrán et al. 2003; Lu et al. 2011). In all three populations, the morphophysiological traits tended to have similar or substantially higher heritabilities under WS than under WW. Secondary traits that are useful in the breeding process for drought tolerance should also be well correlated with GY

(Bäzinger et al. 2000). Significant phenotypic and genotypic correlations were observed between GY and the anthesis-silking interval, ears per plant, stay-green and plant and ear heights. Associations between these traits and GY under drought and optimal water conditions in maize have been previously demonstrated (Bolaños and Edmeades 1996; Ribaut et al. 1997; Messmer et al. 2009; Zheng et al. 2009; Lu et al. 2011). Of the secondary traits evaluated, only ASI and EPP showed consistent increases associated with GY under drought conditions. The strong association of GY with ASI and EPP under drought conditions has been confirmed in a number of progeny evaluation trials of tropical maize under drought stress (Bolaños and Edmeades 1996; Beltrán et al. 2003).

Low levels of plant senescence and high contents of leaf chlorophyll were favourable for high grain yield (Messmer et al. 2011). In our study, stay-green traits demonstrated a moderate to high association with GY under both water regimes. High levels of chlorophyll might even be associated with an increased photosynthetic capacity that is crucial to maintain the continuous flux of assimilates balance in maize (Cai et al. 2012a). Water stress accelerates the remobilisation of protein from senescing leaves, resulting in an inevitable decrease in green leaf area and thus the photosynthetic activity and assimilation of the sugars needed for grain fill (Escobar-Gutiérrez and Combe 2012). A delay in the onset or progress of senescence during grain filling has been positively yield under drought conditions (Bänzinger et al. 2000; Zheng et al. 2009; Lu et al. 2011; Messmer et al. 2011).

The extension and efficiency of the root system is a key factor for the regulation drought adaptation in plants. Deep root systems can access water stored in deep soil layers, extracting more water from soil to maintain yield productions. Unfortunately, studies correlating root depth and drought tolerance in maize are

scarce, mainly especially at the molecular level because root traits are difficult and expensive to measure (Hund et al. 2011). A high correlation between root capacitance and fresh root weight has been demonstrated in sunflower under controlled experiments (Rajkai et al. 2005). However, in our experiments, we measured root capacitance under field conditions, and this trait showed lower heritability and no significant genetic correlation with grain yield. These results are consistent with the results of Monneveux et al. (2008), Messmer et al. (2011) and Lu et al. (2011). Thus, it was reasonable to conclude that root capacitance is not a valuable secondary trait for drought tolerance in maize; therefore, this trait was not included in the QTL mapping studies.

Many efforts have been made to identify the genomic regions responsible for yield and characterise secondary traits under drought and optimal water conditions (Beavis et al. 1994; Ribaut et al. 1997; Lima et al. 2006; Messmer et al. 2009; Zheng et al. 2009; Messmer et al. 2011). The combination of SNP markers, which allow the identification of the physical position of QTLs, with meta-QTL analysis is an important tool to identify the hotspot regions of these traits in order to characterise their relationships under both water regimes and future marker-assisted selection. A significant number of QTLs have been commonly detected either in the same positions or overlapping physical intervals across three different maize tropical populations, which prompted us to perform a meta-QTL analysis using the QTLs identified in single environment analyses under WW and WS. The co-location of the QTLs for these traits potentially reflects the medium to high correlation observed between GY and secondary traits. Of the seven GY-mQTLs included in this study (Chapter 1), five GY-mQTLs overlapped by at least one secondary trait (Table 5, Fig. 1).

A constitutive QTL is consistently detected across most environments, while an adaptive QTL is detected only in specific environmental conditions or increases in expression under the influence of an environmental factor (Collins et al. 2008). QTLs can be categorised according to the stability of their effects across environmental conditions. Almost all mQTLs were integrated using a single QTL under WS and WW conditions, suggesting the constitutive nature of these genomic regions for secondary traits. However, 100% and 75% of the integrated QTLs for ASI and EPP, respectively, were obtained under water-stressed conditions, which revealed an important drought adaptive mechanism associated with these traits. This might explain the significant increase in the correlation of GY with ASI and EPP under WS detected in our study and previous reports (Bolaños and Edmeades 1996; Beltrán et al. 2003).

Two genomic regions on chr.1 and chr.10 harboured overlapping mQTLs for grain yield, stay-green and plant ear height. The region on chr.1 (bin 1.05/06) contained clustered GY-mQTL (161.07-183.29 Mb), SG-mQTL (161.07-183.83 Mb) and PEH-mQTL (~ 119.01-161.08 Mb). On chr.10, the region from 130.59 to 141.82 Mb overlapped with the GY-mQTL (~ 121.49-147.46 Mb), SG-mQTL (111.26-141.82 Mb) and PHE-mQTL (130.59-148.48 Mb). Many earlier studies have reported QTLs for yield and secondary traits in these two regions on chr.1 and chr.10 under optimal and water-stressed conditions. A meta-analysis study involving 17 independent QTL mapping studies with grain yield, flowering trait and plant height revealed meta-QTLs for drought tolerance on bin 1.05/06 at a physical interval of 178.87 to 180.72 Mb and on chr.10 in an interval of 116.24 to 126.70 Mb (Li et al. 2010). Moreover, individual studies that were not included in the meta-QTL analysis discussed above have demonstrated that these regions are important for the regulation

of yield and secondary traits. Zheng et al. (2009) evaluated an $F_{2:3}$ population (Qi-319 x Mo17) and reported one constitutive major QTL for stay-green expressed at 40, 50 and 60 days after flowering at the same physical interval (164.55 to 200.72 Mb at bin 1.06) under optimal water conditions. Using only 160 SSR markers in the CML444xMALAWI population, Messmer et al. (2011) detected one QTL for chlorophyll content at bin 1.06 with a marker peak at approximately 185.06 Mb under WW and WS conditions. Trachsel et al. (2010) evaluated the same population under greenhouse conditions and detected one QTL in bin 1.06 that was responsible for high leaf chlorophyll content and the efficiency of the photosynthetic machinery (Φ_{PSII}) for early vigour. Cai et al. (2012a) evaluated RILs populations from a cross between Ye478xWu312 and detected one QTL on bin 10.04 that was responsible for the regulation of chlorophyll content in ear leaves under optimal and low nitrogen and phosphorus levels. Messmer et al. (2011) confirmed that the genomic region on chr.10 (04-06) plays an important role in the chlorophyll level and leaf senescence under drought and optimal water conditions. Consistently, most of the QTLs detected on bin 10.04-07 for stay-green traits were considered as major QTLs (R^2 > 10.0%). In addition, these two genomic regions on bin 1.06 and 10.04-07 have been reported as constitutive regulators of maize height under abiotic stresses and optimal conditions among temperate and tropical germplasms (Tang et al. 2007; Salvi et al. 2011; Chen et al. 2011; Cai et al. 2012b). Moreover, the genomic region on bin 10.06 has been considered as an important regulator of agronomic traits in maize (Pilu et al. 2011).

Two constitutive genomic regions on bins 4.09 and 5.05 were detected for stay-green traits and grain yield. However, most of the integrated QTLs for both traits were detected under drought conditions, emphasising the importance of stay-green traits under water limitation conditions. In the same study discussed above, Messmer

et al. (2011) detected QTLs for leaf senescence on bins 4.09 and 5.05 under both water regimes. Wang et al. (2012) evaluated one F_2 population under optimal water conditions from a biparental cross A150-3-2 (a stay-green inbred line) and Mo17 (a normal inbred line) and detected one QTL for leaf green area on bin 4.09 at approximately 240.71 Mb at post-flowering. Zheng et al. (2009) detected one significant QTL for stay-green on chr.5, overlapping the genomic region (175.30-199.69 Mb) detected in our study. This previous result confirms the importance of stay-green traits under drought and optimal water conditions for yield production in maize.

In this study we revealed one genomic region on chr.3 (3.06 at 169.75-178.28 Mb), which played an important role in the expression of morphophysiological traits in maize. This is the first study that revealed one genomic region regulating several secondary traits in maize using meta-analysis. The region on bin 3.06 harboured QTLs for all of the traits included in this study (Fig. S1) under drought and optimal water conditions. This finding suggests an important role for the MAS programme because even traits that are not well correlated, genomic region on chr.3 (165.80-178.22 Mb) is responsible for ASI, EPP, SG and PEH. Interestingly, 65% of the integrated QTLs in this region were obtained under WS conditions, which emphasises the adaptive mechanism of this region for drought tolerance. Several independent studies have demonstrated that this region is responsible for the regulation of morphophysiological traits in maize. Salvi et al. (2011) evaluated the BC_5F_4 population from a biparental cross between Gáspe Flint (open-pollinated variety as a donor) and B73 (RIL as a recipient genotype) and revealed one genomic region on bin 3.05-0.7 that was important for the regulation of the number of ears per plant and plant height. Another study using a set of 142 RIL from a biparental cross between

B73xH99 revealed one QTL for plant height on bin 3.06 (Frascarolli et al. 2009). Similarly, using a mixed model approach in a $F_{2:3}$ tropical maize population under abiotic stress (drought or/and low nitrogen) and optimal conditions, Malosetti et al. (2008) detected one region in chr.3 with coinciding peaks for number of ears per plot and plant height. A stable QTL for EH and PH under optimal water conditions based on four Brazilian environments was detected on bin 3.06-0.7 in a yellow tropical maize germplasm (Lima et al. 2006).

Previous reports have shown that the genomic regions on bin 3.06 play an important role in stay-green traits in maize. Zheng et al. (2009) evaluated the maize population described above and detected one genomic region on bin 3.06 at approximately 175.78 to 194.18 Mb that was responsible for the regulation of stay-green traits at 40 days after flowering time. Messmer et al. (2011) reported one QTL on bin 3.06 for leaf senescence under intermediary water stress. Cai et al. (2012) reported one QTL at 169.61 to 190.25 Mb for chlorophyll content in maize under low nitrogen levels and optimal conditions. A meta-analysis involving 12 independent QTL mapping studies with grain yield, flowering trait, plant height, root traits, stomatal conductance, acid abscisic concentration and ear per plant revealed one constitutive mQTL for drought tolerance on bin 3.06 (Hao et al. 2010). Li et al. (2010) conducted another meta-QTL analysis involving seven populations with grain yield, flowering time and plant height traits under drought conditions that were not considered by Hao et al. (2010); these authors revealed one mQTL in an physical interval at 172.70 to 176.20 Mb on bin 3.06. Interestingly, in a meta-analysis involving 15 individual studies under optimal and drought conditions, Hund et al. (2011) demonstrated that this region in bin 3.06 plays an important role in the regulation of root traits (root pulling force and lateral roots). A genome-wide

association study with 1536 SNP chips and a set of 95 inbred lines of parents from the most popular hybrids in China identified one SNP on chr.3 viz., pzb01919.1 (178.23 Mb) that was strongly associated with GY, ASI and the drought tolerance index across different environments (Hao et al. 2011). The physical interval on bin 3.06 delimited to mQTL-ASI, mQTL-SG, mQTL-EPP and mQTL-PEH contained candidate genes, such as *Zmm16* (GRMZM2G110153 - MADS-domain transcription factor), which is implicated in reproductive organ development (Setter et al. 2011), and *psbs1* (GRMZM2G077333_T01 - photosystem II subunit), which acts as a pigment chaperone for the incorporation of chlorophyll molecules into pigment-binding proteins during photoassimilate production (Hankamer and Barber 1997).

The six genomic regions regulating grain yield in three populations each overlapped with at least one secondary trait, which merits attention for their further utilisation in marker-assisted selection within pedigree breeding and population improvement programmes. Taken together, these results imply that the genomic regions with overlapping traits might play important roles in conferring yield advantages under drought stress and optimal environments. Our results provided direct evidence that the GY under stress was genetically associated with all measured morphophysiological traits. However, unless resolved through further fine mapping studies, it would be difficult to conclude whether many QTLs occur as a cluster in this region or if the pleiotropy of a single genomic region is responsible for the manifold effects identified in the current and previous studies. As anticipated from the correlation results, the QTLs from different traits are linked but not well correlated, as demonstrated on bin 3.06; however, some QTL clusters were also trait specific, demonstrating that the target traits considered here have different genetic bases and need to be considered in a complementary manner to improve drought tolerance in

maize. The ten meta-QTL regions identified in the present investigation merit attention for their further utilisation in marker-assisted selection and recurrent selection activities within pedigree breeding and population improvement programmes.

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Supplementary information:

Table S1. Genomics positions, LOD values, genetic effects, gene actions and phenotypic variations (R^2) for detected QTLs in well-watered (WW) and water-stressed (WS) environments on RILs families from CML444xMALAWI. Abbreviations are given in Table 1.

Trait	Treat.	Chr	Pos (cM)	Marker Interval	Physical position[1]	LOD	R^2	Add[2]	Direction
ASI	WW	-	-	-	-	-	-	-	-
	WS	1	267.0	d8.3-bnlg1720	265.20-264.73	3.56	6.01	-0.37	MALAWI
		3	173.0	umc7-umc3b	170.27-192.26	4.55	7.98	-0.42	MALAWI
		5	172.0	pza02209.2-mc48b	180.43-**	2.86	5.50	-0.36	MALAWI
		10	126.0	bnlg236-umc1038	140.96- 148.09	3.35	10.67	0.44	CML444
EPP	WW	1	336.0	pza02359.10-phm9807.9	293.91-294.31	3.78	8.43	0.02	CML444
		3	95.0	phi053-bnlg420	126.51-143.17	3.65	7.25	0.02	CML444
	WS	1	346.0	bnl6.32-bnlg2123	297.96-292.60	3.29	8.33	-0.03	MALAWI
		2	179.0	umc8g-umc135	40.94-41.69	3.38	7.67	0.03	CML444
SENES	WW	-	-	-	-	-	-	-	-
	WS	4	76.0	pza03231.1-pza03409.1	104.16-128.63	7.89	15.76	-0.30	MALAWI
		4	85.0	csu100-umc156a	136.06-150.21	4.66	8.52	0.22	CML444
		8	114.0	phm12749.13-umc48a	155.07-161.78	4.25	8.01	0.21	CML444
		10	67.0	pza00048.1-pza01919.2	98.58-111.26	3.44	6.92	-0.21	MALAWI
CEL	WW	9	127.0	umc113a-umc105a	17.81-18.56	4.03	7.85	0.27	CML444
	WS	6	233.0	phm4468.13- umc2059	167.53-187.77	4.24	9.46	-0.21	MALAWI
CYL	WW	9	128.0	umc113a-umc105a	17.81-18.56	2.63	5.89	0.23	CML444
	WS	3	155.0	umc7- pza03733.1	165.99-180.53	2.70	5.84	-0.17	MALAWI
EH	WW	3	155.0	pza03733.1-umc7	170.27-180.53	5.67	9.47	2.46	CML444
		4	98.0	umc156a-pza00453.2	150.21-166.28	3.50	6.49	2.09	CML444
		6	233.0	umc2059-phm4468.13	167.53-168.77	4.09	6.63	2.05	CML444
		7	10.0	phm4135.15-umc1066	6.44-10.79	3.19	5.52	1.87	CML444
		9	23.0	umc1733-bnl1588	145.34-146.52	5.73	8.17	2.29	CML444
		9	105.0	pza03416.7-umc105a	16.98-18.56	2.82	4.48	1.69	CML444
		10	79.0	umc1115-npi232a	126.260-130.96	3.26	4.76	-1.80	MALAWI
	WS	6	152.0	mmc0241- bnlg1732	145.49-151.96	3.80	5.68	-2.14	MALAWI
		6	214.0	bnlg1740- umc2059	164.86-168.77	3.21	5.06	1.88	CML444
		10	66.0	pza00048.1-pza01919.2	98.58-111.26	5.14	7.88	-2.46	MALAWI
PH	WW	1	73.0	bnlg1627-umc11a	18.92-34.92	2.98	5.26	-2.59	MALAWI
		1	156.0	bnlg1057-umc1122	191.01-201.47	2.79	5.47	2.64	CML444
		6	70.0	pza03069.4-bnl2151	81.81-88.70	5.15	10.18	-3.62	MALAWI
		7	116.0	pza00795.1-pza02373.1	159.42-161.62	6.11	12.03	3.96	CML444
		8	39.0	npi110a-umc103a	10.19-16.95	2.55	3.80	-2.20	MALAWI
	WS	1	88.0	pza00962.1- bnlg439	43.83-43.86	4.78	8.86	-2.65	MALAWI
		6	48.0	umc85a- bnlg426	8.29-16.25	4.20	7.89	-2.53	MALAWI
		8	53.0	bnlg669-phm2350.17	22.66-23.99	2.57	5.39	-2.13	MALAWI

[1]Physical position of the marker flanking the QTL in Mb (10^6 pb). [2]QTLs with additive effects are presented with positive values and were contributed by the parent CML444; the negative values were derived from the parent MALAWI. ** Unknown physical position.

Table S2. Genomics positions, LOD values, genetic effects, gene actions and phenotypic variations (R^2) for detected QTLs in well-watered (WW) and water-stressed (WS) environments on $F_{2:3}$ families from CML440xCML504. Abbreviations are given in Table 1.

		QTL Position					Genetic Effect[2]			Gene Action[3]		
Trait	Treat.	Chr.	Pos. (cM)	Marker Interval	Physical position[1]	LOD	R^2 (%)	Add	Dom	|d/a|	Nature	Direction
ASI	WW	1	653.0	phm1438.34-pza03578.1	212.39 - 252.22	3.14	12.87	0.30	-0.40	1.34	D	CML440
		2	124.0	pza01336.1-phm4880.179	31.39 - 103.49	2.70	9.12	-0.34	-0.12	0.46	PD	CML504
		9	90.0	pza00947.1-pzb01899.1	96.89 - 98.51	3.72	6.44	0.32	-0.16	0.49	PD	CML440
	WS	1	136.0	pza00887.1-pza03521.1	10.07-10.93	2.59	3.50	0.26	-0.18	0.70	PD	CML440
		2	69.0	phm6111.5-pza00590.1	21.98-29.99	5.99	8.10	0.30	-0.43	1.40	OD	CML440
		3	148.0	phm2290.12-phm15449.10	121.88-125.08	3.34	3.42	-0.06	0.37	6.24	OD	CML504
		5	270.0	phm3512.186-pza02099.3	203.43-206.33	5.20	6.57	-0.40	-0.17	0.42	PD	CML504
		7	161.0	pza01542.1-pza02449.13	129.79-134.85	3.12	1.99	0.09	0.27	2.90	OD	CML440
		8	96.0	pza00717.15-pza01257.1	68.48-68.79	3.34	4.41	0.35	-0.01	0.03	A	CML440
		9	118.0	pza02397.1-phm4905.6	133.88-133.92	3.80	5.18	-0.39	0.04	0.11	A	CML504
		9	168.0	pza00323.3-pza00832.1	142.50-147.13	3.21	3.82	0.25	-0.21	0.83	PD	CML504
EPP	WW	1	252.0	pza03189.4-pza01267.3	64.26-76.05	7.90	14.18	-0.01	-0.04	4.11	OD	CML504
		1	275.0	pza03240.1-pza03240.2	90.77-90.78	8.14	14.14	0.01	0.04	4.19	OD	CML440
		2	69.0	phm6111.5-pza00590.1	21.98-21.99	3.45	2.29	0.01	-0.01	0.60	PD	CML440
		6	171.0	pzb01222.1-pza02815.25	164.42-167.88	4.15	5.82	0.01	-0.01	1.21	D	CML440
		7	164.0	pza01542.1-pza02449.13	129.79-134.85	2.89	1.73	0.01	0.01	1.61	OD	CML440
	WS	1	173.0	pza02094.9-pza01030.1	15.72-17.68	4.87	5.59	0.05	-0.23	4.42	OD	CML440
		1	229.0	pza02376.1-phm2130.29	44.51-55.56	4.28	4.29	0.05	-0.21	4.62	OD	CML440
		1	567.0	PZB00008.1-pza01588.1	258.50-268.37	7.66	8.73	-0.03	0.31	10.37	OD	CML504
		3	230.0	PZB01457.1-phm13742.5	212.72-213.61	4.77	5.41	-0.05	-0.24	4.96	OD	CML504
		3	267.0	pza01154.1-pza01688.3	216.03-223.67	2.90	3.64	0.01	0.20	15.54	OD	CML440
		5	236.0	pza00963.3-phm3512.186	203.43-207.27	4.04	4.69	-0.02	-0.24	15.73	OD	CML504

		5	294.0	pza02099.3-pza01680.3	206.33-208.90	11.04	14.22	0.18	0.34	1.90	OD	CML440
		7	98.0	phm10225.15-phm1912.20	155.97-162.17	3.58	3.96	0.06	0.21	3.61	OD	CML440
		7	134.0	pza00405.6-pza03166.1	137.63-138.55	4.45	4.77	-0.06	-0.23	3.58	OD	CML504
		10	156.0	pza01141.1-phm3844.14	120.54-146.55	3.26	4.47	-0.15	0.08	0.53	OD	CML504
SENES	WW	1	151.0	pza03521.1-pza0355.1	10.07-12.21	4.14	6.46	-2.31	-0.47	0.20	A	CML504
		1	530.0	pza03020.8-phm3563.17	284.03-282.04	3.00	4.66	1.06	1.83	1.73	O	CML440
		3	184.0	pza02299.16-pza00920.1	103.38-142.82	3.23	5.12	1.80	0.34	0.19	A	CML440
		5	115.0	phm565.31-phm13675.17	24.24-66.81	3.62	4.46	1.58	-1.14	0.72	PD	CML440
		6	170.0	pzb01222.1-pza02815.25	164.4-167.88	2.74	2.29	-0.97	0.87	0.89	D	CML504
		7	9.0	pza00418.2-pza01210.1	71.72-75.10	2.82	4.29	0.66	-1.84	2.77	OD	CML440
		8	129.0	pza00739.1-pza01049.1	105.80-129.04	3.37	5.50	2.16	-0.04	0.02	A	CML440
		9	173.0	pza00323.3-pza00832.1	142.50-147.13	3.58	5.21	0.69	2.39	3.44	OD	CML440
	WS	1	606.0	pza00381.4-phm14475.7	237.64-245.118	2.75	4.37	-1.88	0.82	0.44	PD	CML504
		3	43.0	pza03527.1-pza02098.2	5.70-8.11	3.96	6.11	2.26	-0.22	0.10	A	CML440
		9	48.0	zhd1.1-pza01999.3	22.04-23.22	3.29	4.38	-1.68	0.95	0.57	PD	CML504
		10	145.0	pza01141.1-phm3844.14	120.54-146.55	3.36	17.81	-3.82	-5.78	1.51	OD	CML504
CEL	WW	3	192.0	pza00186.4-pza01962.12	165.80-178.23	6.02	9.74	-49.29	3.05	0.06	A	CML504
		3	220.0	pza03458.1-pzb01457.1	203.32-212.73	5.50	9.16	49.84	-8.21	0.16	A	CML440
		4	44.0	pza00436.7-phm2159.8	6.40-28.98	5.39	8.99	-1.84	66.57	36.08	OD	CML504
		5	114.0	phm565.31-phm13675.17	24.24-66.81	4.25	5.69	-4.06	52.57	12.95	OD	CML504
		8	168.0	phm4203.11-phm4757.14	133.53-151.45	3.37	4.43	20.64	-29.29	1.42	OD	CML440
	WS	4	52.0	pza03385.1-phm14717.2	37.0-40.52	4.27	7.03	19.82	-9.97	0.50	PD	CML440
		6	133.0	phm4503.25-phm2108.61	161.13-161.66	3.28	5.08	-16.44	8.99	0.55	PD	CML504
CYL	WW	4	114.0	pza03205.1-pza01810.2	202.88-203.77	3.92	2.57	18.00	-0.99	0.05	A	CML440
		5	202.0	phm13696.9-phm13696.11	175.36-175.37	2.53	3.72	-4.37	-29.52	6.76	OD	CML504
		7	162.0	pza01542.1-pza02449.13	129.79-134.85	2.94	2.03	2.91	23.83	8.18	OD	CML440
	WS	1	252.0	pza03189.4-pza01267.3	64.26-76.05	2.80	4.69	-15.49	-5.04	0.33	PD	CML504

		5	176.0	pza01530.1-pza02408.2	37.79-180.41	3.82	5.00	14.92	-3.05	0.20	A	CML440
		5	230.0	pza00980.1-pza00963.3	203.77-207.27	3.12	5.38	-17.67	0.58	0.03	A	CML504
		7	164.0	pza01542.1-pza02449.13	129.79-134.85	3.48	4.24	-1.48	21.53	14.57	OD	CML504
EH	WW	1	190.0	pza01030.1-phm13619.5	17.68-22.03	2.88	4.00	-2.76	0.11	0.04	A	CML504
		2	126.0	pza01336.1-phm4880.179	31.39-103.49	6.72	10.47	0.35	-6.08	17.48	OD	CML440
		7	169.0	pza01542.1-pza02449.13	129.79-134.85	3.80	10.56	0.68	7.44	10.99	OD	CML440
	WS	1	189.0	pza01030.1-phm13619.5	17.68-22.28	7.08	9.61	-2.92	0.45	0.15	A	CML504
		5	153.0	pza00996.1-pza01530.1	37.78-37.79	3.13	3.72	-0.48	-2.34	4.85	OD	CML504
		6	93.0	pzb00414.2-phm15665.22	131.40-137.48	3.38	3.66	1.99	0.68	0.34	PD	CML440
		7	164.0	pza01542.1-pza02449.13	129.79-134.85	3.69	6.21	0.43	3.49	8.14	OD	CML440
		9	79.0	pza01791.2-pza00947.1	77.46-96.89	3.25	3.50	-1.83	-0.36	0.20	A	CML504
		10	146.0	pza01141.1-phm3844.14	120.54-146.55	2.50	4.13	-2.20	0.96	0.44	PD	CML504
PH	WW	1	201.0	phm13619.5-phm4597.14	22.28-38.61	4.87	8.52	-4.91	0.84	0.17	A	CML504
		1	612.0	pza02655.9-phm297.18	217.50-239.31	17.01	30.45	-2.37	11.51	4.86	OD	CML504
		7	49.0	pza02018.1-pza03583.1	86.40-128.40	3.04	4.85	3.47	3.52	1.01	D	CML440
		8	97.0	phm4552.6-pza01257.1	67.93-68.79	4.79	7.38	4.95	0.46	0.09	A	CML440
	WS	1	199.0	phm13619.5-phm4597.14	22.28-38.61	3.44	4.52	-2.37	-0.48	0.20	A	CML504
		1	571.0	pza01588.1-pzb00008.1	258.50-268.37	5.02	6.84	-2.74	1.37	0.50	PD	CML504
		3	187.0	pza00920.1-pza00186.4	142.82-165.80	3.83	5.81	-2.41	1.53	0.63	PD	CML504
		6	94.0	pzb00414.2-phm15665.22	131.40-137.48	3.77	4.51	2.51	-0.47	0.19	A	CML440
		8	102.0	phm4552.6-pza02683.1	67.93-90.84	4.29	5.92	2.90	-0.55	0.19	A	CML440

[1]Physical position of the marker flanking the QTL expressed in Mb (10^6pb). [2]Genetic effects of the QTL are determined by the A: additives and D: dominant effects. QTLs with additive effects are shown with positive values and were contributed by the parent CML440, and QTLs with negative values are from the parent CML504. [3]Gene action determined on the basis of the level of dominance was calculated using the ratio between dominant and additives effects of the QTLs (|d/a|) according to Stuber et al. (1987) criterion: additive (A) = 0 – 0.20; partial dominance (PD) = 0.21 – 0.80; dominance (D) = 0.81 – 1.20, and overdominance OD > 1.20.

Table S3. Genomic positions, LOD values, genetic effects, gene actions and phenotypic variations (R^2) for detected QTLs in well-watered (WW) and water-stressed (WS) environments on $F_{2:3}$ families from CML444xCML441. Abbreviations are given in Table 1.

	QTL Position						Genetic Effect[2]			Gene Action[3]	
Trait	Treat.	Chr.	Pos. (cM)	Marker Interval	Physical position [1]	LOD	R^2 (%)	Add	Dom	|d/a|	Nature	Direction
ASI	WW	1	145.0	pza03578.1-d8.2	252.22-265.19	2.57	1.85	-0.10	0.18	1.80	OD	CML441
		1	571.0	phm595.30-pza02087.2	281.82-284.06	3.28	4.94	-0.15	-0.16	1.05	D	CML441
		3	114.0	phm2423.33-pza00297.2	**-227.68	4.62	7.95	-0.21	-0.29	1.38	OD	CML441
		4	280.0	pza02027.1-pza03459.1	132.98-134.29	2.66	6.27	-0.15	-0.32	2.14	OD	CML441
		5	165.0	pza00963.3-pza02015.11	207.27-207.46	2.87	4.33	-0.19	0.00	0.01	A	CML441
		7	63.0	pza01909.2-pza01210.1	6.44-75.09	3.38	3.30	-0.15	0.17	1.13	D	CML441
		10	96	pza01001.2-phm3736.11	146.54-147.76	3.38	9.20	0.26	-0.19	0.73	PD	CML444
	WS	1	117.0	d8.2-pzb00114.1	265.20-275.98	2.54	1.81	-0.04	0.29	7.06	OD	CML441
		1	571.0	phm595.30-pza02087.2	281.81-284.05	4.24	4.74	-0.35	0.08	0.22	A	CML441
		2	62.0	pza01280.2-phm3668.12	149.43-195.55	4.45	6.76	0.44	-0.08	0.19	A	CML444
		2	262.0	pza01232.1-pza02939.10	155.86-157.15	14.07	20.20	-0.68	0.12	0.18	A	CML441
		3	275.0	pza01154.1-phm2672.19	216.03-219.86	2.57	2.07	-0.16	0.31	1.93	OD	CML441
		4	182.0	phm687.25-phm2159.8	17.48-28.98	3.76	5.10	0.40	-0.13	0.33	PD	CML444
		6	48.0	phm15961.13-pza00355.2	9.56-78.75	2.80	3.52	-0.29	-0.19	0.66	PD	CML441
		6	94.0	pza00214.1-phm12794.47	91.70-128.47	2.99	3.43	0.28	-0.28	1.00	D	CML444
		7	60.0	pza01909.2-pza01210.1	6.43-75.09	2.76	1.63	-0.10	0.32	3.21	OD	CML441
		10	40.0	pza03605.1-phm5435.25	141.83-144.234	2.99	4.12	-0.02	-0.47	30.45	OD	CML441
		10	272.0	phm5740.9-pzb01301.5	8.77-9.75	2.96	2.57	0.27	0.09	0.35	PD	CML444
EPP	WW	1	483.0	pza03183.5-pza03189.4	46.07-64.26	2.71	3.02	0.00	-0.02	5.20	OD	CML444
		3	272.0	pza01154.1-phm2672.19	219.03-219.86	2.74	3.31	0.00	0.02	4.75	OD	CML441
		9	42.0	pzb01110.6-pza01062.1	24.03-88.06	4.41	9.50	-0.01	0.03	3.33	OD	CML441
		10	273.0	phm5740.9-pzb01301.5	8.77-9.75	2.73	2.88	-0.01	0.01	1.03	D	CML441

	WS	2	184.0	pza02264.5-phm13440.13	2.52-3.16	2.53	2.64	0.01	0.00	0.34	PD	CML444
		2	348.0	pza01352.5-pza02170.1	226.45-231.19	3.22	3.83	-0.01	-0.02	3.33	OD	CML441
		3	116.0	phm2423.33-pza00297.2	**-227.68	2.99	1.59	0.00	0.01	6.50	OD	CML444
		4	125.0	pza01905.12-phm2438.28	3.54-244.09	2.71	1.18	-0.01	0.01	1.57	OD	CML441
		8	15.0	pza01857.1-pza01079.1	14.12-156.10	4.67	5.19	0.01	0.01	0.63	PD	CML444
		10	236.0	pza02398.2- pza01141.1	99.47-120.54	4.66	32.28	-0.05	0.04	0.78	PD	CML441
SENES	WW	1	115.0	d8.2-pzb00114.1	265-19-275.98	2.63	1.74	1.59	1.08	0.68	PD	CML444
		1	345.0	pza03200.2-pza02741.1	148.69-161.07	5.13	7.74	3.10	0.41	0.13	A	CML444
		1	425.0	pzb02058.1-pza02195.1	28.52-39.29	3.31	3.92	-2.25	0.07	0.03	A	CML441
		3	212.0	pza00210.9-phm5502.31	29.69-67.28	3.15	4.48	-2.35	0.08	0.03	A	CML441
		4	307.0	fea2.3-pza02194.1	132.73-180.31	2.55	2.13	1.69	0.79	0.47	PD	CML444
		6	52.0	phm15961.13-pza00355.2	9.56-78.75	4.88	7.40	-3.00	-0.44	0.15	A	CML441
		7	57.0	pza01909.2-pza01210.1	6.43-75.09	3.95	7.61	-1.08	4.59	4.24	OD	CML441
		10	101.0	pza01001.2-phm3736.11	146.54-147.76	2.61	2.80	1.26	2.10	1.67	OD	CML444
	WS	1	15.0	phm14475.7-phm174.13	256.24-294.91	3.86	3.70	0.78	-3.33	4.26	OD	CML444
		1	192.0	phm3034.3-pza01921.19	61.31-255.55	2.65	2.18	1.69	1.76	1.04	D	CML444
		1	344.0	pza03200.2-pza02741.1	148.69-161.07	3.82	3.77	2.29	-0.30	0.13	A	CML444
		2	90.0	phm3668.12-phm7953.11	195.55-195.93	10.97	13.25	-3.62	3.82	1.06	D	CML441
		2	295.0	pza02731.1-phm16125.47	197.10-199.41	18.20	21.04	4.93	-0.31	0.06	A	CML444
		5	137.0	pza02015.11-pza03339.2	207.46-210.89	4.09	4.42	0.59	3.34	5.62	OD	CML444
		8	292.0	phm1834.47-pza01316.1	162.44-164.37	2.62	2.64	1.96	0.29	0.15	A	CML444
		10	52.0	pza03605.1-pza03603.1	141.82-141.83	2.93	3.76	0.78	2.78	3.58	OD	CML444
CEL	WW	1	46.0	pzb01227.6-pza00623.3	288.44-293.63	2.55	1.96	15.71	-28.07	1.79	OD	CML444
		1	374.0	phm12323.17-csu1138.4	53.35-119.02	2.88	3.35	-20.24	-39.14	1.93	OD	CML441
		3	115.0	pza00297.2-phm2423.33	**-227.68	2.91	1.58	-2.72	30.46	11.18	OD	CML441
		4	307.0	fea2.3-pza02194.1	132.73-180.31	5.66	9.35	-47.86	16.08	0.34	PD	CML441
		5	367.0	pza02207.1-pza01304.1	49.20-178.58	3.11	2.13	-18.23	-29.23	1.60	OD	CML441

		9	69.0	pzb01110.6-pza01096.1	24.02-133.45	2.70	3.14	-2.02	-44.77	22.19	OD	CML441
		10	147.0	pza01456.2-phm3844.14	135.93-146.55	2.61	4.24	-22.06	42.00	1.90	OD	CML441
	WS	1	14.0	phm14475.7-phm174.13	256.24-294.94	3.41	3.34	-1.45	23.64	16.28	OD	CML441
		1	487.0	pza03183.5-pza03189.4	46.06-64.26-	3.48	4.24	-3.63	-23.44	6.45	OD	CML441
		2	300.0	pza01885.2-pza02418.2	206.88-214.64	3.81	4.83	-17.29	3.62	0.21	A	CML441
		3	226.0	pza00279.2-pza02616.1	52.80-210.16	10.02	15.71	26.65	22.90	0.86	PD	CML444
		5	120.0	pza03167.5-pza03339.2	207.60-210.89	3.87	7.28	-1.60	-32.45	20.31	OD	CML441
CYL	WW	1	47.0	pzb01227.6-pza00623.3	288.44-293.63	3.54	4.35	20.96	-39.32	1.88	OD	CML444
		1	567.0	phm595.30-pza02087.2	281.81-284.05	2.63	2.46	23.29	-16.93	0.73	PD	CML444
		4	308.0	fea2.3-pza02194.1	132.73-180.30	8.18	13.70	-60.33	-5.41	0.09	A	CML441
		5	368.0	pza02207.1-pza01304.1	49.20-178.58	3.00	1.82	-3.27	-35.11	10.75	OD	CML441
		10	105.0	pza01001.2-phm3736.11	146.54-147.76	2.70	3.53	-30.97	15.36	0.50	PD	CML441
		10	143.0	pza01456.2-phm3844.14	135.93-146.55	2.54	3.29	-27.47	20.20	0.74	PD	CML441
	WS	1	44.0	pzb01227.6-pza00623.3	288.44-293.63	2.57	1.25	9.25	6.53	0.71	PD	CML444
		1	487.0	pza03183.5-pza03189.4	46.06-64.26	2.73	1.22	9.92	-11.29	1.14	D	CML444
		2	135.0	phm482.27-pza02727.1	11.10-227.92	3.78	3.73	16.02	-25.03	1.56	OD	CML444
		2	236.0	pza00365.2-pza02337.4	1.22-15.50	7.35	7.58	-26.14	-15.49	0.59	PD	CML441
		2	314.0	pza02418.2-pza01352.5	214.64-226.45	2.64	2.02	-12.54	2.65	0.21	A	CML441
		3	226.0	pza00279.2-pza02616.1	52.80-210.16	16.08	20.71	29.55	35.10	1.19	D	CML444
		4	127.0	phm2438.28-pza01905.12	3.55-244.08	4.31	3.42	15.11	-23.23	1.54	OD	CML444
		5	167.0	pza02015.11-pza03339.2	207.27-207.43	3.84	3.68	19.39	0.64	0.03	A	CML444
		7	68.0	pza01909.2-pza01210.1	6.43-75.09	3.73	2.69	-16.93	3.41	0.20	A	CML441
		8	158.0	pza01210.1-pza01691.1	5.99-11.62	3.97	4.64	14.25	-22.92	1.61	OD	CML444
		10	97.0	pza01001.2-phm3736.11	146.53-147.76	2.71	2.39	-5.97	-20.14	3.37	OD	CML441
EH	WW	1	333.0	pza03200.2-pza02741.1	148.69-161.07	5.57	8.42	3.47	-0.07	0.02	A	CML444
		4	4.0	pza01477.3-pza01187.1	172.30-177.67	6.21	9.86	3.37	-3.33	0.99	D	CML444
		10	270.0	phm5740.9-pzb01301.5	8.77-9.75	4.42	6.88	-2.04	2.96	1.46	OD	CML441

		10	316.0	phm15868.56-pza02527.2	137.13-148.49	2.64	2.43	-1.78	-2.11	1.19	D	CML441
	WS	1	310.0	pza02467.10-phm5622.21	183.83-196.92	6.52	8.39	3.10	-0.61	0.20	A	CML444
		2	25.0	phm6111.5-pza01374.1	21.99-28.31	3.93	4.30	2.14	0.93	0.43	PD	CML444
		3	212.0	phm5502.31-pza00210.9	29.69-67.28	3.83	4.09	2.09	-0.08	0.04	A	CML444
		4	98.0	pza00529.4-pza03322.5	240.76-242.02	6.15	6.77	2.18	1.25	0.58	PD	CML444
		5	127.0	pza03167.5-pza03339.2	207.60-210.89	2.74	2.83	1.46	1.22	0.84	PD	CML444
		7	62.0	pza01909.2-pza01210.1	6.43-75.09	5.26	7.13	2.29	1.33	0.58	PD	CML444
		10	208.0	pza02320.1-pza02398.2	99.47-132.25	3.89	8.01	-3.67	-0.54	0.15	A	CML441
PH	WW	2	251.0	pza02337.4-pza02450.1	15.51-47.58	4.76	7.03	3.45	-0.16	0.05	A	CML444
		2	276.0	pza03692.1-pza02890.4	166.65-187.22	2.90	3.98	-0.84	3.30	3.93	OD	CML441
		3	42.0	pza01447.1-pza00363.7	53.55-132.19	2.81	4.13	3.13	0.12	0.04	A	CML444
		4	81.0	pza02779.1-phm5599.20	207.11-239.23	4.21	6.31	2.98	1.24	0.42	PD	CML444
		6	223.0	pza02478.7-phm5529.4	141.11-167.12	3.17	11.95	-3.12	5.28	1.69	OD	CML441
	WS	1	48.0	pza00623.3-pzb01227.6	288.44-293.63	2.81	2.23	1.03	1.49	1.44	OD	CML444
		1	367.0	csu1138.4-phm12323.17	53.35-119.01	5.92	7.70	2.74	0.89	0.33	PD	CML444
		2	177.0	pza02264.5-phm13440.13	2.52-3.16	4.97	5.80	2.37	0.09	0.04	A	CML444
		2	350.0	pza01352.5-pza02170.1	226.45-231.19	3.41	2.40	1.46	-0.14	0.10	A	CML444
		3	210.0	phm2343.25-pza02255.2	27.98-33.22	5.10	5.97	2.50	-0.50	0.20	A	CML444
		4	273.0	pza03459.1-pza02027.1	134.29-132.97	5.41	9.92	-0.36	-4.74	13.20	OD	CML441
		5	256.0	ae1.7-pza00881.1	97.98-167.87	7.11	10.98	-0.36	4.76	13.08	OD	CML441
		5	308.0	pza03340.2-pza00222.7	20.20-58.57	4.37	5.92	-0.81	-2.96	3.67	OD	CML441
		7	68.0	pza01909.2-pza01210.1	6.43-75.10	5.89	7.89	2.76	0.26	0.09	A	CML444
		9	71.0	pzb01110.6-pza01096.1	24.03-133.45	2.63	1.72	-1.30	0.23	0.18	A	CML441

[1]Physical position of the marker flanking the QTL expressed in Mb (10^6 pb). [2]Genetic effects of the QTL are determined by the A: additives and D: dominant effects. QTLs with additive effects are shown with positive values and were contributed by the parent CML444, and QTLs with negative values are from the parent CML441. [3]Gene action determined on the basis of the level of dominance was calculated using the ratio between dominant and additives effects of the QTLs (|d/a|) according to Stuber et al. (1987) criterion: additive (A) = 0 – 0.20; partial dominance (PD) = 0.21 – 0.80; dominance (D) = 0.81 – 1.20, and overdominance OD > 1.20.

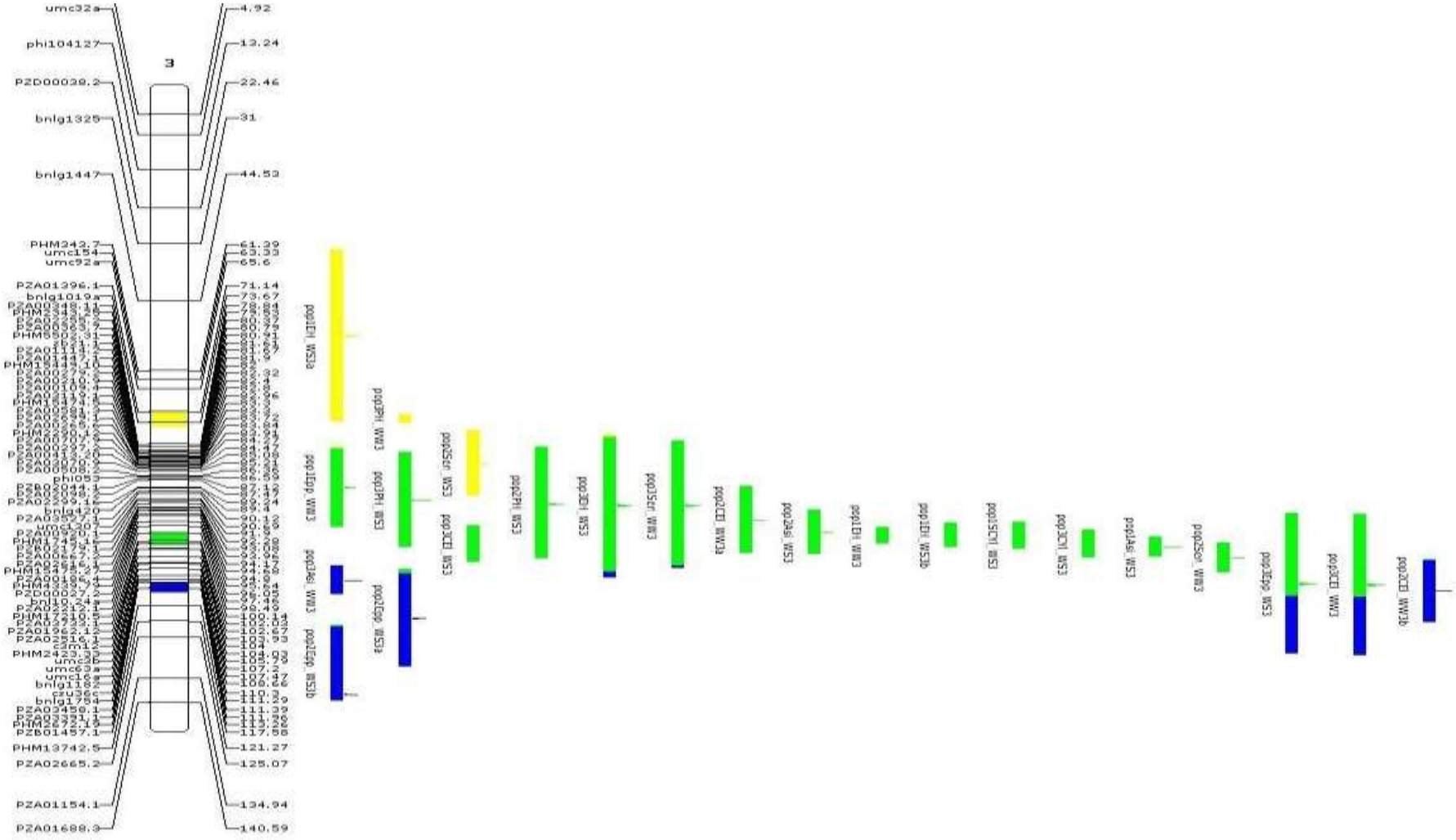

Figure S1. The meta-QTL analysis revealed a hotspot genomic region on chromosome 3 (green area) for morphophysiological traits related to drought tolerance in maize.

4. GENERAL CONCLUSION AND OUTLOOK

Climate change is predicted to increase the likelihood of drought stress in agriculture. This adversely can increase the often expose and extent of period of water limitation in rainfed maize cultivation. Therefore, crop improvement for limited-water condition is a priority for plant breeding programs worldwide. At the same time, efforts to impart drought tolerance should not result in compromised GY under optimal conditions, which requires identification of genotypes that equally perform well under WS and WW conditions. However, conventional breeding for drought tolerance in maize is slow and selection based solely on grain yield could be inefficient. Only a multidisciplinary approach combining conventional breeding, physiology and biotechnology can reveal the genetic basis of the complex physiological and morphological responses of maize to water-limited conditions.

This problem has already been identified for a long time in CIMMYT, in consequence a long term focus exists on the development of drought tolerant germplasm. In the present study, we have identified several families within three tropical biparental populations that combine high yield potential under WW environments with good tolerance to WS conditions, which could serve as an excellent source of initial source population for marker-assisted recurrent selection in the tropical breeding programs.

The metaQTLs regions identified in the present investigation merit attention for their further utilization in the marker-assisted selection as well as marker-assisted recurrent selection activities within pedigree breeding and population improvement programs. Most part of QTLs regulating yield was overleaped by secondary traits that can increase the selection efficiency for drought tolerance. All these results imply that the genomic regions with overleaped trait identified may play important roles in

conferring yield advantages not only under drought stress but also in optimal environments. Our results provided direct evidence that GY under, mainly stress were genetically associated with all measured morphophysiological as anthesis-silking interval, ears per plant, stay-green and plant and ears height. However, unless resolved through further fine mapping studies, it would be difficult to conclude whether many QTLs occur as a cluster in this region or pleiotropy of single genomic region is responsible for the manifold effects identified in the current and previous studies. As anticipated from the correlation results, QTL from different traits are linked, even not well correlated as demonstrated on bin 3.06, but a couple of QTLs cluster were also trait specific, demonstrating that target traits considered here have different genetic basis and need to be considered in a complementary way to improve drought tolerance in maize.

Successful applications of the large number of QTL data generated during the last two decades, however, are scarce. This fact is due weak associations between markers and target QTLs, interactions of QTLs with the environment, the lack of large stable QTLs for grain yield and sensitivity of the QTLs to the genetic background. This was the first study that combined information from three biparental maize tropical populations and SNP information. This molecular makers allowed identified specific genomic physical position, which was harbored several drought related genes. This approach may play an important role in the efficiency of MAS for manipulating QTLs for polygenic traits. Because the accumulation of QTLs in some genetic regions across environments and different population, as observed in this study, corresponded to the results of other published studies on drought tolerance in maize. These hotspot regions that regulate yield and morphophysiological traits

related to drought tolerance in maize merit attention for their further utilization in the marker-assisted selection.